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L. Cuénot

Nancy, 4 avril 1935

LUCIEN CUÉNOT[1]

BECAUSE of the originality and fruitfulness of his work, the rare quality of his erudition, his critical exactingness and keenness, the breadth and discrimination of his thought, Lucien Cuénot merits first rank in contemporary French biology.

Cuénot was born in Paris, quartier des Batignolles, October 21, 1866. His father, a humble postal employee, had a lively curiosity for the things of the mind which impelled him to study at the Sorbonne and the Collége de France after retirement.

The young Lucien, having received a scholarship after his certificate of studies, entered the lycée Chaptal, where his remarkable talents became manifest. The inclination toward natural science was fixed at that time. He collected insects, frogs, and plants, and searched for fossils, during his walks. Bachelor, then student at the Sorbonne, Cuénot's enthusiasm was aroused in a course by Lacaze-Duthiers, and he turned toward zoology. After qualifying for the licentiate degree in 1885, he presented a brilliant doctorate thesis in 1887 on the Asteroidea. Nominated at once as *préparateur* in anatomy and comparative physiology at the Faculty of Sciences of Paris, he began the study of medicine, but abandoned it the following year to accept a call to Nancy as *Maître de Conférences* (1890). Here he served an apprenticeship in histology with Professors Prenant and Nicolas. Beginning in 1898, he occupied the chair of zoology at Nancy, where he lived out his entire career as professor and scientific investigator, preferring the tranquility of Lorraine to the allurements of the capitol. It was here in 1890 that he married Mlle. Geneviève de Maupassant, his six children were born, and that he died, rich in honors, January 8, 1951.

Lucien Cuénot was made a corresponding member of the *Institut de France* in 1918, and became a non-resident member in 1931.

A first class naturalist, Cuénot excelled in interpreting organic structures, and discerning affinities between organisms, in clarifying our understanding of existing forms by comparison with their fossil relatives. He gave due importance to the details of nomenclature without losing sight of the broad perspectives of general zoology. He was the opposite of the narrow specialist and gave his attention successively to groups far removed from each other, the Protista, Echinodermata, Sipunculoidea, and the Tardigrada.

Cuénot contributed to the better understanding of the fauna of Lorraine and the Arcachon basin by describing several new species, correcting erroneous names, and in making more precise various details of ethology and ecology. He revealed, by observations which remain classic, the details of the sexual cycle in the Gregarina; he investigated by ingenious and elegant methods absorption and excretion in various invertebrates; and he studied reflex bleeding of certain insects, regeneration of the antenna in phasmids, caudal autotomy among rodents, homochromy, etc.

Turning to sex determination, Cuénot contributed an important memoir in 1899 embodying personal observations and critical comments. At that stage the problem seemed a single one. Nothing was known about chromosome mechanisms, and

[1] The retiring editors of Genetics, who arranged with M. JEAN ROSTAND for this biographical note, are indebted to NICOLE and WALTER PLAUT for a translation of the article from the French.

several writers still maintained that the fertilized egg was sexually indifferent and that the sex of the individual depended upon exterior influences brought to bear during the course of development.

Cuénot exposed larvae of Lepidoptera, Diptera and Batrachia to very dissimilar diets and showed that neither quantity nor quality of food given the young altered the sex ratio significantly. He concluded that sex determination was precocious, being realized in the fertilized egg, and that it involved entirely unknown internal factors.

Cuénot proceeded naturally from the study of sex to that of heredity. The suggestion was made to Mlle. Barthelet, one of his students who had requested a subject for experimental study, that she undertake cross-breeding between gray and white mice to test an old and tenacious idea among breeders that a first-used male could have an effect on subsequent broods (telegony). While guiding the efforts of his student Cuénot soon realized the advantages of the mouse for the experimental study of heredity with respect to ease of management, fecundity, and variety of color types.

Now, it was precisely at this moment (1900) that the Mendelian revolution burst with the sensational publications of Hugo de Vries, Correns, and Tschermak. Were Mendel's laws valid for the animal as well as the plant kingdom? This was the question which Cuénot posed and to which he quickly (1902) gave a positive answer, based on the mouse investigations, at about the same time as William Bateson in England recognized Mendelism in the guinea pig and the fowl.

Other naturalists before Cuénot—von Guaita, W. Haacke, and Coladon, in particular—had crossed gray and white mice, but not having the guiding thread of Mendelism, they could not arrive at any general conclusion.

Cuénot did not limit himself to verifying the classical Mendelian facts in mice. He brought to light an assemblage of new facts which formed the beginning of our understanding of factor or gene interaction and also of multiple allelism.

He showed, in effect, that albino mice (entirely lacking coat color) can transmit different hereditary potentialities for pigmentation. Among several albino mice which have been crossed, for example, with black mice one mouse will produce black, another gray, and another spotted, offspring. Notwithstanding phenotypical identity these albinos differ in genotype for pigmentation. The color factors cannot come to expression because there is present in every albino mouse a certain "conditional" factor of such a nature that no kind of pigment can be produced. As Cuénot said "Albinism is a mask" which can cover great genetic diversity. This was an important finding because it revealed the first example of factor interaction.

The discovery of lethal genes was of still greater consequence. In 1905 Cuénot hit upon the paradox of a strain of mice—the yellow strain—which resisted all attempts at purification. It was impossible through the application of Mendelian methodology to isolate a stock which produced yellow individuals exclusively, whereas in theory the union of two impure yellow mice should produce a certain proportion (¼) of pure yellows as the result of combination of "yellow" gametes from mother and father.

To account for this impossibility, Cuénot supposed that the pure yellow combina-

tion was not formed because a female gamete containing the yellow factor could not be fertilized by a male gamete containing the same factor. This "selective fertilization" hypothesis was erroneous, and it was observed later that the yellow-yellow combination regularly is formed but is incompatible with normal development, all pure yellows dying in the uterus. Cuénot's researches on yellow mice afforded the first example of genetic lethality, which came to occupy eventually such a prominent place in the study of plant, animal, and human heredity.

It is curious, moreover, to note that, proceeding from a mistaken hypothesis, Cuénot unwittingly had made a correct prediction, namely, that there would be found in the progeny of impure yellow mice two yellows to one non-yellow, which conforms to the experimental finding. (E. B. Wilson and Punnett had commented that on the Cuénot hypothesis there would be formed three yellow to one non-yellow.)

Let us also mention, touching further on genetics, Cuénot's researches on the inheritance of piebald spotting, the experimental production of atavism starting from individuals of known hereditary composition, the non-transmission of naphthalene-induced cataracts (in collaboration with R. Lienhart), and the inheritance of cancer predisposition (in collaboration with Mercier).

Finally, let it be recalled, he was one of the first to suggest a comparison between the activities of genetic factors and enzymes.

The merit of Lucien Cuénot in this domain is enhanced by the fact that his work originally was misunderstood or, in any case, was not properly appreciated in his own country. Cuénot fought alone for many years in France against a body of opinion adhering obstinately to old Lamarckian concepts and which denied any general significance to Mendelism.

Lucien Cuénot did not participate directly after 1912 in genetics, which was to rise so brilliantly with the work of the American school (Morgan, Bridges, Sturtevant, Muller, Painter, and others) on Drosophila, the fruit fly. Doubtless it is to be regretted that the great scientist did not persevere in the path which he had helped to open; but Cuénot continued to serve biology on the theoretical plane by reflecting fruitfully on problems of evolution and organic adaptation. If this part of his work is more open to attack than that which preceded it, it attests nevertheless to an originality of views and a critical sagacity which even his adversaries respected.

Stating freely that, as concerns the fact of evolution, he had never felt "the insidious bite of doubt", Cuénot did not deign to look on the anti-evolutionist attitude as anything but incompetence or bad faith. He devoted himself to making the idea of evolution concrete—with reference at least to the animal kingdom—as a "family tree" which he did not present as definitive but as one which appeared to him to be consistent with existing zoological knowledge. Cuénot inserted thirty buds or leaves on the trunk and branches of his tree, corresponding to the phyla, or "*clades*." The two most recent among these, vertebrates and arthropods, date back at least 500 million years which, according to Cuénot, indicates clearly that the process of "*cladogenèse*" is irrevocably terminated on the globe. Life, he said, has known its period of youth and creativity, characterized by an intense production of "*groupes cladiques*"; it has now come to a state of maturity and relative sterility. Nevertheless, evolution—and even macroevolution—has not stopped but continues within the

preexisting "*clades*", where new types can arise continuously (by neoteny, for example), and maintain themselves if they find an empty niche which offers a favorable milieu.

In explaining evolution of species Cuénot could not but take into account the considerable stock of knowledge from genetics. Like every naturalist of today, he ascribed a major role to mutation, which is the only mode of hereditary variation of which we now have concrete evidence; and his profound knowledge of genetic processes enabled him to interpret in terms of genes and chromosomes a good number of species-forming mechanisms.

Seeing in over-all adaptation (as to aquatic, aerial, or subterranean environment) "a major question of evolution", he tried to explain the harmony between form and environment by the idea, which he personally brought forward, of preadaptation. According to him, the environment does not give rise to the appearance of adaptive characters; it simply offers favorable conditions for the survival and reproduction of mutants which presented these characters beforehand.

Differing from orthodox neo-Darwinists, he disputed the constructive omnipotence of natural selection; he insisted on the fact that "death is not the differentiator in nature". Nevertheless, he granted that selection operates among mutants more or less capable of resisting a climatic accident, exploiting a nutritive resource, or providing defence against a predator or parasite.

Cuénot showed a certain repugnance toward entering the debate concerning the transmission of somatically acquired characters. Agreeing that we have no evidence for the phenomenon and that all experience has been insufficient to furnish a demonstration he recalled, and not without some desire to be accommodating, particular embryological data, as among the "uncertainties of biology", which seemed to support the Lamarckian thesis, notably ontogenetic facts concerning "preparation for the future", for example, the embryo presents characters in a causal relationship to the conditions of adult life (thickening of the sole of the foot in man, callosities in the dromedary, wart hog, and ostrich), or, for example, in which it shows a *coaptation* between two parts which, not being contiguous in the embryo as they will be in the adult, cannot be molded, one upon the other (coaptation in the boar between the canine and the upper lip, and between the head and the forelegs in the phasmid, etc.).

But, in truth, and we here touch the very foundations of his thought, Lucien Cuénot did not believe that mutation, even supported by a grain of Lamarckism, could explain the totality of evolution. An "unsatisfied mutationist", as he called himself, he contested the view that accidental mutations, chosen through natural selection, could result in the development of complex organs such as the eye, the brain, or the wing.

The emptiness of explanations based on fortuities seemed to him to be particularly manifest in the case of the little devices which abound in living organisms and which, by their finished appearance, their mechanical perfection, and their adjustment to an end, lead inevitably to the thought of *tools* made by man.

Cuénot discerned an intention, an "anti-chance" element, in many natural devices: The parachutes of the Composite fruit or of the gliding animal, the swimming oars of the water beetle, the anchor of the Synaptidae, the hooks of the Ophiuroidea, the valve traps of the Utricularia, the suckers of the cephalopods and leeches, injec-

tion tubes, illuminating apparatuses, electric batteries, music boxes, folding knives, pressure buttons, and the like. He saw in the objective and exhaustive study of these "natural tools" the means of reviving, in the light of morphological analysis, the old comparison between vital industry and human industry so much exploited in theological finalism.

This "*faculté artisane*" whereby living and inert matter is essentially distinguished, which is never merely that of a geometer or painter, Cuénot connects with a kind of mysterious inventive power which exerts itself in the germs themselves and is capable of guiding or orienting the mechanical effects of chance. But his thinking on that point is not often so fluid and equivocal; now he sees in germinal invention a simple property of living protoplasm analogous to irritability, assimilation, organization, regeneration; and then he seems to want to make of it an immaterial agent, transcendant, reminiscent of a metaphysical world, but perhaps "more real than that which we touch and deduce."

Regardless of one's judgment on this refined neofinalism, which recalls somewhat Claude Bernard's *idée directrice*, one must clearly understand that Cuénot had no sympathy whatever with the naive providential finalism of Bernardin de Saint Pierre or of Paley. In the last period of his existence Cuénot, whose thinking evolved until the hour of his death, declared himself as a broad, monistic pantheist. "Pantheism is free and has nothing of theology .... I take as metaphysical ornaments the differences between the pantheism of Spinoza and the materialistic pantheisms of Holbach, Diderot, and the moderns. The scientists are the priests, the researches are the prayers."

In truth, no precise categorization would fit this skeptical, discriminating and independent spirit which, in confronting the enigmas of philosophical biology, employed itself in marking the places of our ignorance rather than in proposing illusory solutions.

Cuénot's work encompasses a large number of specialized publications, some monographs and syntheses: *Moyens de défense dans la série animale* (1892), *Influence du milieu sur les animaux* (1894), *La genèse des espèces animales* (1911, 1921, 1932), *L'Adaptation* (1925), *L'Espèce* (1935), *Introduction à la génétique* (in collaboration with J. Rostand, 1935), *Invention et finalité en biologie* (1941), *L'Evolution biologique* (in collaboration with A. Tétry, 1951). This last and most important work of all represented in some way his intellectual testament.

Cuénot collaborated also in several collective works (*Traité de zoologie* by Pierre P. Grassé, *Traité de paléontologie* by Piveteau, etc.).

His books are admirably composed and organized. The documentation, always at first hand, is neatly and concisely presented, without vain dialectics, and with scrupulous objectivity and a constant concern to establish the line between the demonstrated and the hypothetical, the compelling certitude and the possible interpretation. The style, well turned, somewhat nervous, often enhanced by a humorous touch, is that of an accomplished writer.

Cuénot was a teacher without peer. His students are unanimous in paying respect to the vigor of his instruction, which strove less to fill minds than to mold them.

He did not disdain the application of his pedagogical and expository skills to the

popularization of natural science. He contributed much by conferences and occasional articles to the awakening among the public of a taste and respect for biology.

Cuénot gave much time to the Museum of Natural History in Nancy, of which he made an incomparable teaching instrument, portraying visually in vivid fashion the great problems which occupied him: heredity, evolution, and adaptation.

He had a lively interest in the history of religions and on questions of exegesis. One can read in the *Revue Scientifique* under the pseudonym S. Lazare some pages of his on miracles. He battled incisively and with courage and persistence against the pseudosciences, psychic research, divination, astrology, and the like. He even thought of devoting to those subjects a complete volume in which he, as a good positivist, would bring to trial certain perennial and dangerous forms of modern credulity. Here again Cuénot gave proof of his good intellectual taste.

Highly literate, Cuénot continuously re-read his favorite authors, Anatole France, Henri de Regnier, Wells, and Kipling, and entertained himself by making amusing sketches along the margins of the text.

Sometimes critical of his colleagues, Cuénot was indulgent with serious amateurs, and welcomed the young. His friends will never forget his lively conversation, mischievous whims, insatiable curiosity which, after 80 years had passed, demanded only "qu'à se mettre à l'école", nor his relaxed but slightly censorious temperament, and his playfulness, so little professorial.

The history of science will remember the name of this eminent biologist who, being competent to observe, experiment, and reflect, perhaps was one of the last able to maintain liaison between nature and the laboratory.

JEAN ROSTAND
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EFFECTS OF THE HOMOZYGOUS MINUTE-IV DEFICIENCY ON THE DEVELOPMENT OF DROSOPHILA MELANOGASTER[1]

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THE Minutes comprise a series of dominant factors discovered by BRIDGES and MORGAN in 1923. The phenotype of all heterozygous Minutes is characterized by short, slender bristles and a longer developmental period than the wild type. In some cases, there may be secondary effects such as smaller body size, somewhat rougher eyes, thinner wings with a tendency toward plexate venation and sterility or low fertility, particularly in the female. All Minutes are lethal in the homozygous condition (BRIDGES and BREHME 1944). In addition, certain Minutes have been found to increase the frequency of somatic segregation and crossing over (BRIDGES 1925; MORGAN, BRIDGES and STURTEVANT 1925; STERN 1936).

These dominant, homozygous lethal factors frequently have been found to be chromosomal deficiencies for one or several loci and salivary chromosome bands. In some cases, the salivary chromosomes appear normal and genetic tests have proven no deficiencies for known genes. In general, however, most authors suggest that such Minutes are deficient for extremely small chromosome regions, not visible in the salivary chromosomes. A summary of the Minute types found and analyzed can be obtained from BRIDGES and BREHME (1944).

In studies of the effects of certain Minutes upon developmental rate, DUNN and MOSSIGE (1937) found that a delay occurred only during the larval period. Their work was concerned principally with three Minutes, *M(3)w*, *M(3)33j* and *M(2)l*^{2}, whose effects on bristle size reduction varied in severity. It was found that the effect on the bristles was inversely proportional to the length of the delay in the larval stage. They concluded that a retardation in growth rate was responsible for the phenotype.

BREHME (1939, 1941a, 1941b) investigated the developmental delay of *M(3)w*, *M(3)Fla* and *M(2)l*^{2} and again determined that retardation occurred in larval life. Even though life was prolonged in early stages, the adult body size was found to be significantly smaller than wild type. At the end of the larval period, the Minutes exceeded the normals in length, but were smaller in width. The wing area of experimental and control flies was also determined. In general, the wings of the mutant types were smaller than those of the controls. BREHME suggested that the effect was due to a reduction in cell size, rather than in cell number. It was also postulated that the prolonged development of the Minutes was not merely a simple slowing-down process but was a symptom of an underlying qualitative difference from the normal.

[1] From a dissertation submitted to the Graduate Faculty of the University of Missouri in partial fulfillment of the requirements for the degree of Doctor of Philosophy. This work was conducted while the author was an Atomic Energy Commission Predoctoral Fellow in the Biological Sciences of the National Research Council.

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Other studies by BREHME (1941c) have employed the transplantation technique in an effort to determine whether or not host tissue can affect developmental rate. When optic discs of *M(3)w* and wild type larvae were implanted into host larvae of the same or alternate genotype, no increase occurred in eye facet number of the transplant when located in a host of a different growth rate. It was also found that the facet number of *M(3)w* was not significantly different from normal, although the area of the individual facets was smaller in the Minute. This last evidence is in accord with the results on wing size (BREHME 1941a).

SCHULTZ (1929) carried out a genetic study of several Minutes in an effort to analyze the developmental process involved. He investigated various combinations of different Minutes and could observe no cumulative effects on such characteristics as viability, female partial sterility, rate of development or general appearance. This fact suggested that the primary reactions causing the Minute phenotype were qualitatively different in different Minutes.

In summary, certain significant facts concerning the Minutes should be emphasized. These numerous factors, many of which are visible deficiencies, have been found to be located in all four chromosomes. Furthermore, the Minutes are mimics in phenotype, for all are characterized by short bristles, delayed development, dominance over wild type when heterozygous and lethality when homozygous. Yet despite these pronounced similarities, many Minutes have been shown to be qualitatively different.

This investigation is the first of a series whose purpose is to determine whether or not the causes of lethality in different homozygous Minutes are related to one another. The present study is concerned with the analysis of the factors which lead to the death of embryos homozygous for a specific fourth chromosomal deletion, Minute-4 (*M-4*).

Minute-4 appeared spontaneously and was first described by MORGAN, STURTEVANT and BRIDGES (1926). Cytological analysis by MORGAN, BRIDGES and SCHULTZ (1934) and BRIDGES (1935) showed that the deficiency chromosome lacked at least four and probably more bands between the line G and the spindle fiber region. BRIDGES and BREHME (1944) report the loss of about ten lines from the *M-4* chromosome. Genetic studies by MORGAN, STURTEVANT and BRIDGES (1928), MOHR (1933) and MORGAN, BRIDGES and SCHULTZ (1934) have shown this chromosome to be deficient for the loci of the mutants *ar*, *ci*, *gvl* and *Scn* but not for *bl*, *ey* or *sv*. The seriation of genes in chromosome IV has recently been studied by FUNG and STERN (1951) and by STURTEVANT (1951) and a revised map of this chromosome has been presented by SLIZYNSKI (1944).

METHODS

The stock used in the present study was originally obtained from the laboratory of DR. A. H. STURTEVANT. Minute-4 heterozygotes, with a background of Stephensville wild type, were maintained by continuous inbreeding and from such individuals the desired homozygotes were obtained.

All eggs were collected and incubated at 25°C on paraffined paper spoons smeared with well-yeasted media. In most cases, females were allowed to oviposit for one-hour

periods although in some instances a shorter interval of time was used. The ages of the embryos were calculated from the midpoint of the laying period.

Embryos were dechorionated in Chlorox, rinsed twice in distilled water and fixed in hot (65° to 70°C) Kahle's fluid (KAHLE 1908). During fixation, the eggs were pricked individually with a very sharp needle. Following the usual dehydration and embedding procedures, embryos were serially sectioned at 6μ and stained with Heidenhain's iron-alum hematoxylin.

A study of the normal embryonic development was first carried out in the Stephensville wild type stock. Approximately 200 embryos were used in this work and were collected and prepared as described above. For the investigation of *M-4* homozygotes, over 3000 embryos ranging in age from fertilization to hatching stages were prepared and examined.

RESULTS

The control animals used for comparison with the lethals were the normally developing sibs of these individuals. These normal animals were of two different genotypes: two thirds were heterozygous for the Minute deficiency and one third was homozygous wild type. In sectioned material, these were indistinguishable from one another.

In order to prove that the individuals studied were the *M-4* homozygotes, a tabulation was made of all developing embryos 16 through 22 hours in age. These stages were chosen to eliminate all subjective factors since abnormal morphology is unmistakable in later embryonic life. Of the total of 769 developing embryos (16–22 hours), 578 or 75.2%, were normal in morphology while 191 or 24.8%, were abnormal. Since all of the defective individuals showed the same deviations from the wild type condition, there can be little doubt that the lethality observed was due to the homozygous presence of the *M-4* deficiency. It should be noted that approximately 10% of the sectioned eggs had failed to develop and were judged to be unfertilized from their characteristic appearance. This was expected since egg counts carried out at the beginning of this study had indicated that approximately 35% of the progeny produced by a cross between heterozygotes failed to hatch.

The Minute-4 homozygotes cannot be distinguished from controls until after ten hours of incubation. As development reaches this point, the normal embryo exhibits the following morphology: Upon contraction of the germ band, the nervous system can be easily identified since it has lifted from the ectoderm and the supraesophageal and subesophageal ganglia are well formed. Proliferating endodermal strands extend from the end of the long tube-like stomodeum. Contraction of the germ band has brought the anus to its definitive posterior position and has resulted in the appearance of a characteristic loop of the hindgut. This organ stretches some distance forward from the anus and then bends posteriorly. From the inner end of the hindgut, the posterior midgut rudiments extend ventrolaterally making contact with the proliferating stomodeal endoderm. Malpighian tubules have been formed by evaginations from the hindgut. The somatic mesoderm has segmented and has begun to move dorsally. Elongation of cells to form the body musculature is in progress ventrally

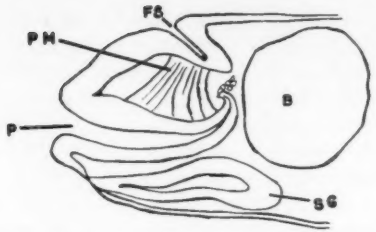

FIGURE 1.—Diagram of the anterior end of a 20 hour *M-4* homozygote illustrating the abnormal foregut and associated structures. B, brain; FS, frontal sac; P, pharynx; PM, pharyngeal muscles; SG, salivary gland.

and laterally. Splanchnic mesoderm enveloping the outer regions of the midgut rudiments is beginning its differentiation. Just after this stage, involution of the head occurs with the consequent rolling inward of cells which later take part in foregut and frontal sac differentiation (fig. 2). A complete description of normal ontogeny has been given by POULSON (1950).

The abnormal condition of the homozygous *M-4* embryos first becomes recognizable at the time of yolk enclosure, after completion of 10 to 11 hours of development. These individuals can be distinguished by the relatively large number of yolk spheres which are not included in the midgut (fig. 3). The anterior, middle and posterior midgut rudiments unite at the proper time in the abnormal embryos, but apparently much yolk is left outside for it fills up most spaces between developing organs. In the normal animal, some yolk is always found in the far anterior and posterior regions, but these granules are soon utilized and disappear. Extension of the midgut dorsally to enclose the yolk occurs more slowly than is the case in controls and in some regions the wall of this organ is incomplete, with the consequent escape of yolk.

At this same stage of ontogeny, a second major failure in development can be recognized in the foregut region where the head does not undergo complete involution. During this stage in the control, a rolling-in of ectodermal cells, especially those located dorsally, occurs. This movement carries the cells destined to give rise to pharyngeal and frontal sac structures to their definitive locations. In the *M-4* homozygote, involution begins but fails to be completed. The result is the partial differentiation of a small, defective cephalopharyngeal apparatus located in a short, protruding bulge at the anterior end of the embryo (figs. 1, 3, 5). The frontal sac is represented by a small invagination just dorsal to the cephalopharyngeal structures. With failure of frontal sac formation, none of its derivatives such as mouth hooks or sclerotized plates are formed in later stages.

According to SONNENBLICK (1939 and 1950) and POULSON (1950), and as verified in the present study, the salivary glands originate from paired invaginations on the anterior ventrolateral surface of the embryo. As the stomodeum deepens, the cells of this region move inward carrying with them the openings of the salivary glands. The invaginations for these structures approximate and become united around the tenth hour of development. The distal regions differentiate into the secretory portions of the gland, while the proximal united section develops into the common duct which opens via the hypopharynx into the buccal cavity.

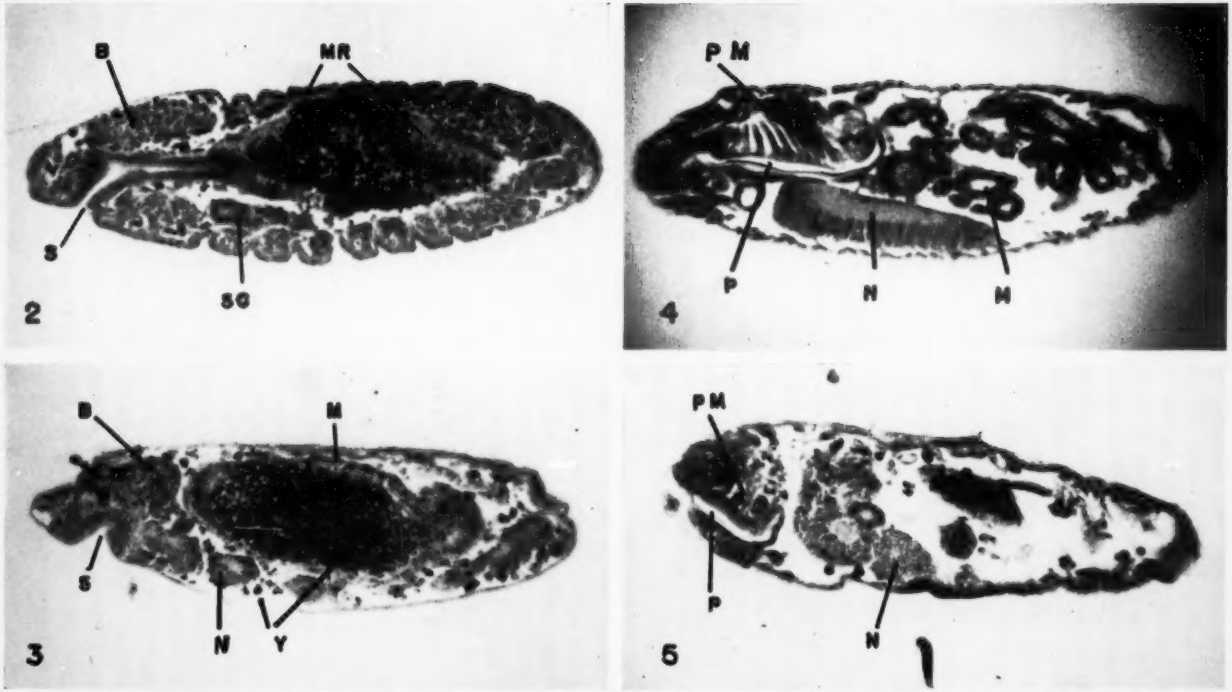

FIGURE 2–5—All figures are oriented with the dorsal surface toward the top of the page and the anterior end to the left. B, brain; M, midgut; MR, midgut rudiments; N, ventral ganglia; P, pharynx; PM, pharyngeal muscles; S, stomodeum; SG, salivary gland; Y, yolk.

FIGURE 2.—Sagittal section of an 11 hour normal embryo showing foregut and midgut formation.

FIGURE 3.—Sagittal section of a 13-hour *M-4* embryo illustrating abnormalities of the foregut, midgut and ventral nerve cord.

FIGURE 4.—Sagittal section of a 21 hour normal embryo showing cephalopharyngeal apparatus, condensed nerve cord and elongate midgut.

FIGURE 5.—Tangential sagittal section of a 21 hour *M-4* embryo showing abnormal cephalopharyngeal apparatus and nervous system.

In the *M-4* homozygotes, the formation of the salivary glands is entirely normal. However, although the movement of ventrolaterally located cells into the stomodeal opening is initiated around nine hours of development, it is never completed. As a result, the salivary glands retain their opening on the external ventral surface of the embryo immediately posterior to the mouth (fig. 1). The separate openings of these structures do fuse, but a true duct does not differentiate. The separate glandular portions develop immediately posterior to this opening and are thus more anteriorly located than is the case in controls. The assumption of secretory activity, as judged by the presence of heavily staining material in the lumen of the gland, occurs at the usual time (10 to 11 hours) in both controls and lethals.

During the period of yolk enclosure and head involution, the esophagus of the *M-4* homozygotes fails to become established and is represented by a few cellular strands which disappear altogether in later stages (figs. 1, 5). Cells which normally contribute to the lengthening of the stomodeum are derived from those which move to the interior of the embryo along with the salivary gland ducts. It is possible that the absence of these cells is related to the developmental failure of the esophagus.

An additional outstanding deviation from the normal condition involves the nervous system and especially the ventral nerve cord. In the middle region of the embryo, the ganglia seem compressed between the sac-like midgut and the ventral hypodermis. Yolk granules can be found above, below and between the ganglia and, indeed, in all body spaces. By 12 hours of development, the ventral nerve cord is strikingly abnormal in that a variable number of ganglia are lacking or are represented by only small masses of neuroblasts (fig. 3). At the same stage in the control, the ganglia are easily visible and can be counted since the nerve fibers in the interior of each stand out sharply. Sufficient nerve fibers differentiate in the abnormal individuals so that these ganglia can also be counted, although the neuropile is usually disorganized. Generally, the first through the third or fourth, and the eighth or ninth through the eleventh pairs of ventral ganglia are present. The interganglionic connectives, well established in the normals at this stage, are usually present toward the anterior and posterior regions of the ventral nerve cord, but missing in the middle portions. It is interesting to note that all ganglia are produced in an apparently normal manner in earlier stages.

As a consequence of head involution in the normal organism, the brain is moved backwards towards the midgut. Posterior displacement of the brain pulls the subesophageal ganglion forward (POULSON 1950). The possible relationship between these movements and the subsequent condensation of the ventral nervous system is unknown. In the Minute homozygote, however, the brain fails to reach its definitive location due to incomplete head involution (fig. 5).

After 16 to 18 hours of development, the lethal form exhibits the same peculiarities as before but with a few modifications. The ventral nerve cord has begun its customary condensation, although only those ganglia between which connectives have been established take part in this movement. Typically, three to four of the ventral ganglia remain at the posterior end of the embryo. Those ganglia which do concentrate anteriorly maintain their distinct outlines since their nerve fibers do not form a single mass as in the controls (figs. 4, 5).

The anterior region of the midgut lies close behind the brain, which in turn is pressed to the posterior end of the abnormal cephalopharyngeal apparatus. Although an anterior constriction of the midgut typically occurs, cardia and caecae are not formed. A very large amount of yolk remains within the midgut and the lengthening process goes on much more slowly than in the control animals. Some regions are lined with cells more cuboidal than is usually the case. In other portions, the cellular lining appears to have given way altogether, releasing the yolk into the hemocoel. It also seems likely that at least some of the yolk found scattered through the body spaces is released by the developmental failure of the esophagus. The visceral musculature of the entire digestive tract is generally normal.

Other differences from the control also become readily evident in the 16 to 18 hour lethal embryo. The somatic musculature which was entirely normal in its segmentation and arrangement in earlier stages fails to complete its differentiation. Apodemes are only weakly developed and although elongate cells are present in the usual muscle arrangement, these fail for the most part to become well established. Indeed, in 18-hour embryos, somatic muscle fibers appear to be rounding up into multinucleate masses and losing their original connections with the body wall. Hypodermal cells form a thin undifferentiated layer beneath these muscles, and the formation of the exoskeleton is observed only in very late stages.

The fat bodies are very difficult to identify and may be represented by a number of cells which can be found clinging to yolk in the hemocoel. These latter cells might also be interpreted as yolk cells released from the midgut.

Germ cells within their mesodermal sheath are usually present in the lethal homozygotes, evidence that certainly a portion of the lateral mesoderm undergoes differentiation. No count of the number of germ cells present was made.

The dorsal vessel and its associated structures could not be identified with certainty. Union of cardioblasts normally occurs during 11 to 12 hours of development at the time of dorsal closure of the embryo. The dorsolateral mesoderm which includes the presumptive cardioblasts, pericardial cells and cells of the dorsal diaphragm is present in earlier stages, but union of cardioblasts and establishment of the dorsal vessel and associated structures is not completed. In some sections, small groups of cells resembling the heart could be found, but they did not extend the length of the embryo and yolk spheres were frequently associated with them. It would seem reasonable to assume that the potentialities for the differentiation of the dorsal vessel are present, but that this structure, like the esophagus and cardia, fails to become established.

The ring gland, composed of corpora cardiacea and corpora allata, could not be located as a definite structure in late stage embryos. Presumably the corpora cardiacea are formed initially since in all early embryos before contraction of the germ band the typical invaginations from the roof of the stomodeum can be found. Cells which would comprise the corpora allata are normally derived from those located at the innermost tip of the frontal sac invagination (POULSON 1950). In the *M-4* homozygotes, formation of the frontal sac is incomplete. Although a small cluster of cells can be found at the posterior end of the abnormal cephalopharyngeal apparatus, the possible homology of these cells with the corpora allata has not been determined.

The absence of the aorta and the disturbed relationships in the anterior region of the embryo make the positive identification of these structures difficult.

The hindgut and Malpighian tubules differentiate in a manner comparable to the controls. It should be noted, however, that the hindgut normally undergoes relatively little differentiation after 12 hours of development when compared to other rapidly developing structures. The Malpighian tubules appear to be normal, although their length has not been measured nor has the onset of functional activity been studied.

Trachae are formed at the usual time and the intimal lining of the larger tubes can be discerned before 18 hours of development. The posterior spiracles, however, are never fully differentiated although union with the lateral tracheal trunks occurs.

At 21 hours of development, the lethal embryo shows little change from the condition previously described. The midgut is usually longer and contains less yolk. In all abnormal embryos, however, the midgut fails to attain the length characteristic of the controls. In the foregut region some differentiation of muscle occurs in the formation of the pharyngeal dilator muscles, prominent features of the normal animal (figs. 4, 5). Only a partial cephalopharyngeal apparatus is formed in the defective organisms and the muscle arrangements are not perfect, although they can be readily recognized. Chitinization of the abnormal foregut is completed by 18 to 20 hours of development. The general shape of the aberrant embryos also becomes somewhat different from that of the normal. Large spaces within the organism result in a sac-like appearance and segmentation becomes quite indistinct. No movements on the part of the abnormal individuals have been observed to occur.

It is difficult to state the exact time of death of the *M-4* homozygotes. These individuals do not hatch and in most instances acquire a brownish coloration, characteristic of autolysis, within 36 hours after fertilization. It is probable that death occurs at slightly different times in different individuals.

DISCUSSION

To clarify the results it is necessary to determine whether or not the various defects of the homozygous *M-4* embryo are related to one another. A search for some basic explanation to account for an array of morphological anomalies has been successfully carried out in a number of cases of developmental failure such as the frizzle fowl (LANDAUER and UPHAM 1936), the Creeper chick (HAMBURGER 1942), the bleb anomaly of the mouse (PLAGENS 1933; BONNEVIE 1934) and the gray lethal mouse (GRÜNEBERG 1936, 1938). Since the presence of yolk in the body spaces is one of the earliest irregularities of the abnormal organisms, it could be considered the cause of other later appearing defects. Studies of early stages have shown that the nervous system first develops normally. The smaller size of the ventral ganglia and the subsequent loss of those located beneath the bulging midgut could be attributed to pressures exerted by the abnormally located yolk spheres. Such obstruction, occurring as it does at the time of nerve fiber formation, might result in suppression of the interganglionic connectives and interfere with their complete separation from the superficial ectoderm.

An interpretation of the anomalies of the *M-4* embryos in terms of abnormal pressures is, however, inadequate because it fails to account for the generally normal

condition of the hindgut, Malpighian tubules and gonads. These organs are also subjected to any pressures exerted by the unenclosed yolk but their development is essentially normal. The period of initial formation of these structures occurs before ten hours of development, the time at which abnormal morphology becomes apparent.

The presence of unenclosed yolk in the body spaces of the abnormal individuals, however, reflects an underlying physiological derangement. In the normal embryo, the inclusion of yolk within the midgut removes most of the nutrient materials from the immediate vicinity of other tissues. What little remains outside soon disappears, a fact which shows that tissues other than gut epithelium are capable of yolk degradation at least for a short time. The abnormal embryos, on the other hand, are characterized by the presence of much unenclosed food which later fails to be metabolized. This observation suggests that the tissues outside the gut of the homozygous *M-4* embryo have lost their capacity for yolk utilization at the time when such yolk rapidly disappears in the control. This condition is permanent, for all abnormal embryos through 22 hours of development show large amounts of yolk outside the midgut walls. In addition, the mesenteron cells seem unable to break down the included food at a rate comparable to that of the control.

Any interference with a crucial phase of metabolism would be likely to result in widespread detrimental effects in a rapidly developing embryo. Such effects could easily bring about a general retardation of growth or a disturbance in normal correlations. Tissues undergoing morphogenesis or differentiation at the time when such interference became effective would be injured to a greater extent than those growing more slowly. CHILD (1925) has shown that embryonic regions characterized by high levels of activity are particularly sensitive to deleterious agents. In chick embryos, the regions first affected by the introduction of metabolic inhibitors are those undergoing rapid changes (SPRATT 1950). STOCKARD (1921) has pointed out that the development of each organ is preceded or initiated by a period of activity which is greatly in excess of the developmental rate of the rest of the embryo. DUNN (1940) has emphasized the fact that anomalies are often produced by a reduction in growth rate. The various parts of an embryo may respond differently to such a delay according to their respective rates of development. The type of abnormality resulting would be dependent on the general pattern of embryonic growth and the time at which any abnormal interruption or alteration of metabolism became effective. Lack of, or incomplete differentiation of, some part could thus be produced by a failure to reach some level or degree of development. The action of a number of lethal factors in Drosophila has also been explained in terms of differential responses on the part of developing organs to a generalized growth restriction (HADORN 1948; GLOOR 1945, 1947).

One outstanding characteristic of the phenotype of all heterozygous Minutes is the pronounced developmental delay in larval stages (DUNN and MOSSIGE 1937; BREHME 1939). Even though early life is prolonged, the body size of Minute larvae and adults is significantly smaller than that of controls (BREHME 1939, 1941a). Reduction in size of the bristles of the adult has been found to be proportional to the increased duration of larval life (DUNN and MOSSIGE 1937). This evidence suggests that growth restrictions in larval life modify the normal tempo of larval de-

velopment and particularly affect rapidly developing, and therefore sensitive, imaginal tissue. In the heterozygous Minute, however, growth restriction is not so severe as to prevent further differentiation.

Since a delay is found in the development of heterozygous Minutes, it is reasonable to postulate that an early and severe derangement in differentiation and metabolism accounts for the death of homozygous Minutes. If such an hypothesis of severe growth restriction is applied to the *M-4* homozygote, the observed anomalies can be better explained and traced to this one derangement.

The period between 10 and 12 hours of development is one of intense activity. The anterior and posterior midgut rudiments fuse and extend laterally and dorsally to enclose the yolk. Dorsal closure of the body wall is effected along with fusion of cardioblasts and formation of the dorsal vessel. In the foregut region, movement of cells into the stomodeum occurs. This shift carries inward to their final location the external openings of the salivary glands whose common duct forms almost at the same time. Invagination of the frontal sac, accompanied by definitive localization of the brain and subesophageal ganglia is completed by 12 hours. The formation of nerve fibers and interganglionic connectives as well as condensation of the nervous system begins around the tenth hour of development.

These complex movements, which occur very rapidly and within a relatively short period of time, must be preceded and accompanied by a very high level of metabolism. It is significant that the structures involved in these activities are the very ones in which anomalies become apparent. Evidently at this stage of ontogeny, the presence of the deleted genetic material becomes essential for continued normal development. A generalized growth restriction first becoming effective at this period would greatly interfere with the localization and differentiation of these structures. Such a generalized metabolic action might be brought about by a number of different physiological circumstances such as insufficient amounts or a total lack of essential enzymes or substrates.

It has previously been pointed out that utilization of yolk by the abnormal embryos is seriously impaired after the time of yolk enclosure. Thus, substrates resulting from yolk degradation must be available only in small amounts beyond this period. Since development is normal up to the time of yolk enclosure, it can be assumed that adequate amounts of substrates have been supplied to and utilized by the various regions of the developing embryo.

It could be suggested that the midgut is the organ primarily affected by the loss of genic material. The further differentiation and eventual functioning of this structure might be so impaired that other regions of the embryo would be affected through lack of nutrients. This explanation is not acceptable in view of the fact that yolk outside of the midgut fails to be metabolized by the tissues of the homozygote, while in the normal animal, utilization of unenclosed yolk by other tissues readily occurs. Therefore, the midgut is only one of many structures severely harmed by some underlying physiological anomaly. Any damage to the midgut which retards its differentiation will eventually contribute to the general anomalous condition of the embryo, but such a consequence is secondary to an initially widespread restriction in development. Whether or not inadequate utilization of substrates present in the yolk represents a physiological anomaly basic to all others is not known at the present time.

SUMMARY

1. The abnormal development of homozygous Minute-4 embryos of *Drosophila melanogaster* was studied and comparisons with the normal animal were made.

2. Control animals consisted of the normally developing sibs of the Minute-4 homozygotes and were of two genotypes: wild type and Minute-4 heterozygotes. In sectioned material through 22 hours of development, these were indistinguishable from one another.

3. The aberrant embryos die in the egg stage within 24 to 36 hours after fertilization. Minute-4 homozygotes cannot be distinguished from controls until after ten hours of development. Major deviations from the normal condition include the following:

Although the midgut rudiments unite at the proper time, dorsal extension of the walls of this structure occurs more slowly than in controls. Subsequent lengthening and coiling is greatly retarded and cardia and caecae fail to appear. Fragility of the walls of the midgut results in the release of yolk into the body spaces. The midgut and hemocoel remain crowded with this material even in late stages.

Abnormalities of the foregut consist of incomplete involution of the head with consequent aberrant formation of the frontal sac and cephalopharyngeal apparatus. The salivary glands retain their opening on the external surface of the embryo. The esophagus fails to become established and disappears entirely in late stage embryos.

The ventral nerve cord is characterized by the loss of a variable number of ganglia, usually the fifth through seventh. In addition, certain of the interganglionic connectives fail to develop and, therefore, three to four pairs of ganglia remain posteriorly when condensation of the nervous system occurs. Nerve fiber regions persist as distinct structures and do not become united into a single central mass as in controls.

The dorsal vessel fails to develop and the presence of the ring gland is questionable. In addition, the somatic musculature undergoes incomplete differentiation and the body segmentation becomes indistinct.

4. The abnormalities of the Minute-4 homozygotes are interpreted in terms of a generalized growth restriction occurring at a critical period in ontogeny.

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EFFECTS OF HOMOZYGOUS FIRST, SECOND AND THIRD CHROMOSOME MINUTES ON THE DEVELOPMENT OF DROSOPHILA MELANOGASTER[1]

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THE Minutes of *Drosophila melanogaster* include a large group of factors which are dominant over wild type when heterozygous, and lethal when homozygous. All heterozygous Minutes are characterized by the presence of short, slender bristles in the adult and by a retardation in development in larval life. Although the Minutes mimic one another in expression, they have been found to occupy a variety of loci in all four chromosomes and are considered by most authors to be chromosomal deficiencies (BRIDGES and BREHME 1944). The behavior of these factors when combined with modifiers or with other Minutes has indicated that many are qualitatively different (SCHULTZ 1929).

The purpose of the present study is to determine whether or not different Minutes, so similar in heterozygous phenotype, also resemble one another with respect to homozygous phenotype. This investigation is concerned with an analysis of the factors responsible for the death of individuals homozygous for seven different Minutes, one of which, *M(1)o*, is located on the X chromosome; two, *M(2)l* and *M(2)l*^{2}, are overlapping deficiencies of the second chromosome; while the other four, *M(3)w*, *M(3)124*, *M(3)B* and *M(3)B*^{2}, comprise an allelic series of the third chromosome. Some information concerning these different Minutes is available in the literature and will be reviewed briefly in the following section.

Minute (1)o resembles other members of the Minute series in that heterozygous females possess smaller bristles and a longer larval developmental period than the wild type. This mutant is lethal in males. Salivary chromosome studies have shown the locus to be between bands 14B1–2 and 15E7 (BRIDGES and BREHME 1944).

Minute (2)l appeared spontaneously and salivary chromosome analysis by BRIDGES has shown that the chromosome is deficient for bands 58A–F (BRIDGES and BREHME 1944). Studies by LI (1927) have demonstrated that the *M(2)l* homozygote dies in the egg stage. According to LI, heterozygous *M(2)l* females regularly lay eggs which are abnormal in appearance not only in regard to shape but also with respect to the character of the yolk, which he describes as glassy in appearance, rather than opaque white. In the present study, similar abnormalities in egg shape were observed, but differences in the appearance of the yolk were not noticeable in living, developing embryos. It should be noted, however, that unfertilized eggs, as well as partially developed embryos, frequently appear quite transparent 24 or more hours after egg deposition. DUNN and MOSSIGE (1937) have reported that a two-day delay occurs during the larval stage of *M(2)l* heterozygotes although the final body size of the

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Minutes is generally smaller than that of wild type sibs. The presence of this mutant is considered by these authors to result in a retarding rather than an extending effect upon larval life.

Minute (2)l^2 is regarded as a deficiency, although salivary analysis by BRIDGES has shown no detectable abnormalities. The locus is associated with band 58F and is thus included within that of *M(2)l* (BRIDGES and BREHME 1944). A developmental delay in larval life is characteristic of *M(2)l²* as reported by DUNN and MOSSIGE (1937) and by BREHME (1939 and 1941a). According to BREHME, Minute male larvae are about 9.9 and female larvae 13.3 hours behind their wild type sibs in puparium formation. A slight lengthening of the pupal period was recorded by DUNN and MOSSIGE (1937). Body size of the adult is significantly smaller than that of controls, when tibia length is used as an index (BREHME 1939). In addition, the surface area of cells of wing epithelium has been found to be smaller than that of wild type. From this evidence, BREHME (1941a) has suggested that a reduction in cell size may account for the smaller body size of the Minutes. The death of *M(2)l²* homozygotes occurs in the first larval instar (BREHME 1939). Homozygous larvae grow only very slightly and do not molt. These observations are confirmed in the present study.

Of the four allelic third chromosome Minutes, *M(3)w* has been considered the most extreme, while *M(3)124* lies between *M(3)w* and *M(3)B* and *M(3)B²* in degree of expression (BRIDGES and BREHME 1944). The growth of larvae heterozygous for *M(3)w* has been investigated by DUNN and COYNE (1935), DUNN and MOSSIGE (1937) and by BREHME (1939, 1941a and 1941b). BREHME reports a 42-hour delay in puparium formation as compared to the wild type. Although the relative growth curves of heterozygotes are practically identical with controls, adult body size is smaller. The transplantation of eye disks of *M(3)w* heterozygotes to wild type larvae has shown that the more rapid growth rate of the host has no effect on the growth rate of the transplant. Facet number of adult Minutes is not significantly lower than that of controls, but the size of individual facets is smaller (BREHME 1941b). Minute (3)w homozygotes have been found to die in the first larval instar and BREHME (1939) has reported a general necrotic condition of larval tissues in these individuals.

METHODS

All stocks used in the present study were originally obtained from the laboratories of either DR. A. H. STURTEVANT or DR. M. DEMEREC. Heterozygotes on a background of Canton-S wild type were maintained by continuous inbreeding for over twenty generations and were the source of the homozygotes studied.

Eggs were collected at hourly intervals at 25°C and prepared as previously described (FARNSWORTH 1957). The ages of all eggs and larvae were calculated from the midpoint of the laying period. Over 500 embryos were studied for each of the Minutes here considered.

With the exception of *M(2)l*, which is lethal in the egg stage, almost all of the homozygotes of the other six Minutes died in larval life. In each of these stocks, however, there were always a few individuals which failed to hatch. In view of this fact, it was necessary to make certain that only one lethal was being considered and that

the total homozygotes, hatched or unhatched, did not exceed the 25% expected from a cross between heterozygotes. To verify this point, a number of egg collections were initiated and the subsequent history, from hatching through eclosion, of every egg laid within a given period was followed and recorded. All unhatched eggs were dechorionated and examined and, if development had occurred, these individuals were fixed and prepared for histological study. Hatched larvae were isolated, counted and classified as to normal or lethal type and transferred to fresh media. Those placed in the normal group included heterozygotes and the homozygous wild type, a classification confirmed upon eclosion. The individuals designated as lethals were placed in small dishes of fresh media and examined daily for evidence of growth or molting. In all such experiments, the number of lethal larvae plus the individuals which had undergone development, but had not hatched, was very close to the 25% expected. With respect to the adults, approximately two thirds were Minute in phenotype, while one third was wild type. From this evidence, it was concluded that the only lethal factor involved in each instance was the specific Minute being investigated.

RESULTS

Of the seven different Minutes here considered, only *M(2)l* homozygotes die in the egg stage. All other Minutes are lethal in the first larval instar. Since these latter forms are almost identical in phenotype, they will be considered together, while *M(2)l*, which represents a much more extreme departure from the normal condition, will be described separately.

Minute (2)l

The Minute (2)l homozygotes cannot be distinguished from their wild type sibs until after 12 hours of development. Just before and during this stage in ontogeny, a number of rapid changes involving complex cell movements normally occur. Anteriorly and ventrally, the rolling inward of cells contributing to the stomodeum proceeds while, dorsally, involution of the head with frontal sac formation is occurring. The anterior and posterior midgut rudiments have joined and are extending laterally and dorsally to enclose the yolk, a process usually completed by around 12 hours of development. Dorsal closure of the body wall with subsequent formation of the heart and aorta proceeds concurrently. For a complete description of these stages, the reader is referred to POULSON (1950).

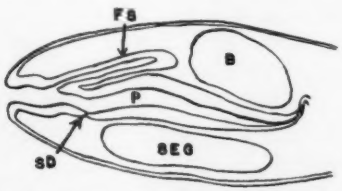
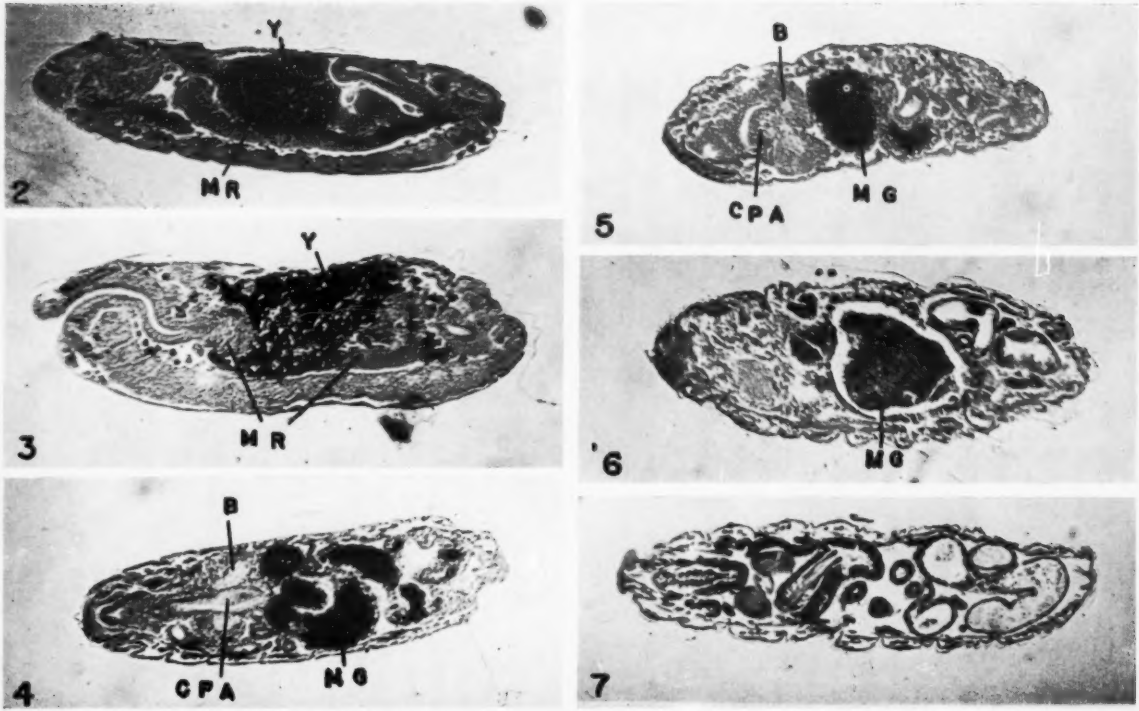


FIGURE 1.—Diagram of a sagittal section of the anterior end of a 16-hour *M(2)l* homozygote showing relationships of frontal sac and pharynx. B, brain; FS, frontal sac; P, pharynx; SD, opening of salivary duct into pharynx; SEG, subesophageal ganglion.



FIGURES 2–7—All figures are oriented with the dorsal surface to the top of the page and the anterior end to the left. B, brain; CPA, cephalopharyngeal apparatus; MG, midgut, MR, midgut rudiment; Y, yolk.

FIGURE 2.—Longitudinal section of 12-hour normal embryo. Midgut rudiments have fused and are enclosing yolk.

FIGURE 3.—Longitudinal section of 12-hour *M(2)1* homozygote illustrating abnormal anterior and posterior midgut rudiments.

FIGURE 4.—Frontal section of 18-hour normal embryo. Midgut is lengthening and cephalopharyngeal apparatus is well formed.

FIGURE 5.—Frontal section of 18-hour *M(2)1* homozygote showing abnormal pharyngeal apparatus and midgut.

FIGURE 6.—Frontal section of 24-hour unhatched *M(2)1*[2] homozygote. Midgut is unsegmented and structures of the anterior end are disorganized.

FIGURE 7.—Frontal section of hatched homozygous *M(2)1*[2] larva.

The first detectable morphological difference between the *M(2)l* homozygotes and the controls is found in the formation of the midgut. The anterior, and to a lesser extent, the posterior endodermal rudiments extend into the yolk as a blunt mass and fail to spread out adequately to enclose this material (figs. 2 and 3). Fusion of these rudiments is thus delayed and, consequently, midgut formation is retarded as compared to the controls. Although the yolk is eventually surrounded, the mid intestine remains abnormal throughout the remainder of development. The walls are thin and in various regions yolk can be found escaping from the gut into the body spaces.

A second major anomaly concerns the foregut. The frontal sac is invaginated only over the anterior end of the pharynx with the result that the pharyngeal dilator muscles are formed in a shortened region. Apparently, invagination of the head is not carried to completion in the *M(2)l* homozygotes for, if the frontal sac were normal in its spread over the dorsal surface of the pharynx a much more extensive cephalopharyngeal apparatus would be formed (figs. 1, 4, 5). The movement of cells into the stomodeum occurs normally, however, and the ducts of the salivary glands reach their definitive location and fuse.

In the Minute homozygote at 16 hours, further abnormalities of the midgut become evident. The cardia differentiates slowly into an organ which is reduced in size and more elongate than that of the controls. Although caecae appear as bud-like evaginations from the mid intestine, they remain very small and fail to achieve normal length. The midgut itself lengthens slowly, and anterior portions in particular remain rounded, sac-like and crowded with yolk (fig. 5). In contrast, the hindgut with appended Malpighian tubules is essentially normal.

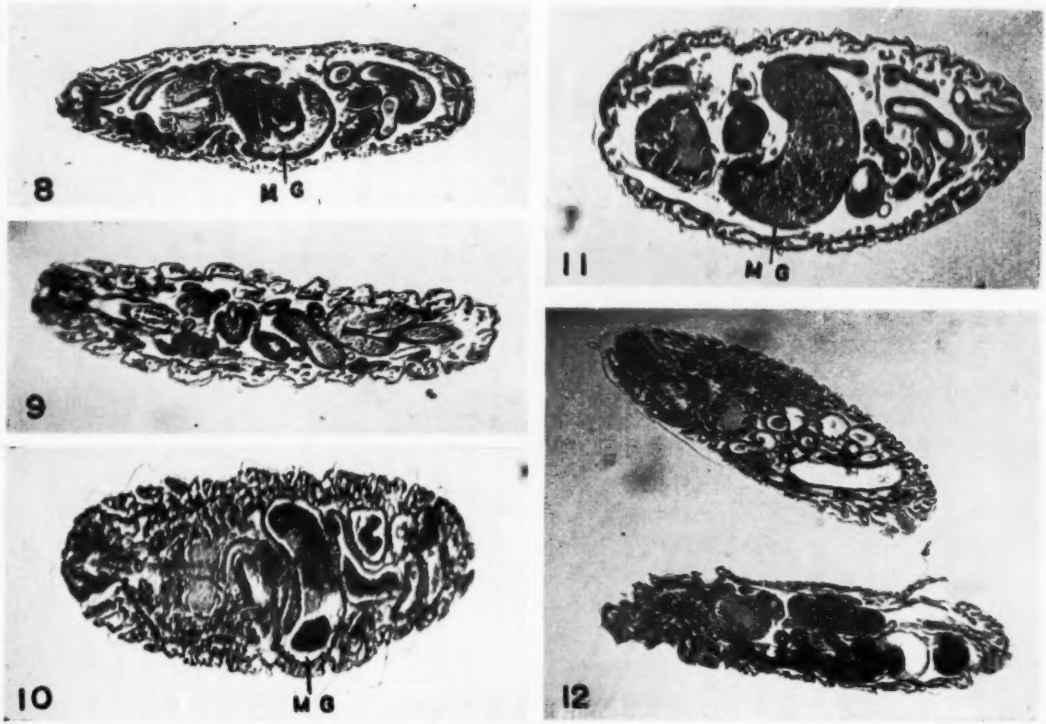
The brain and ventral nerve cord develop in a manner comparable to that found in the controls. Some disorganization in the neuropile is evident in late stages and complete condensation of the ventral ganglia is not usually achieved. However, no ventral ganglia are missing and nerve formation generally proceeds normally.

Other structures, such as the dorsal vessel, gonads, and the very small fat bodies can usually be identified although the presence of the ring gland is not certain. Longitudinal tracheal trunks and their developing side branches are generally normal.

Twenty to twenty-two hour *M(2)l* homozygotes present an appearance similar to that described above. Although the cephalopharyngeal apparatus remains abnormal in position and extent, the various sclerotized plates and hooks of the mouth armature do develop. The midgut does not attain a length comparable to that of controls and not only is yolk withdrawal greatly retarded, but this material frequently can be found scattered through the hemocoel. The *M(2)l* lethal embryo, in general, greatly resembles *M-4* homozygotes with respect to the pattern of abnormalities although the anomalies are not so extensive.

Larval lethals

Almost all homozygotes of *M(1)o*, *M(2)l*^{2}, *M(3)w*, *M(3)124*, *M(3)B* and *M(3)B*^{2} hatch within 22 to 26 hours after egg deposition. Of the few homozygotes which do not escape from the egg, a number are fully developed and are often found to be moving within the vitelline membrane 30 or more hours after normal hatching time. Other unhatched, but developed, embryos present a number of anomalies upon



FIGURES 8–12—Unless otherwise stated, all figures are oriented with the dorsal surface toward the top of the page and the anterior end to the left. MG, midgut.

FIGURE 8.—Frontal section of 24-hour unhatched *M(1)o* homozygote.

FIGURE 9.—Frontal section of hatched homozygous *M(1)o* homozygous larva showing yolk in gut.

FIGURE 10.—Frontal section of unhatched 22-hour *M(3)w* homozygote. Midgut is slow to lengthen and contains much yolk.

FIGURE 11.—Frontal section of 22-hour *M(3)124* homozygote showing large unsegmented midgut.

FIGURE 12.—Top. Sagittal section of 22-hour normal embryo, oriented with anterior end to left and ventral side to top of page. Bottom. Sagittal section of 22-hour unhatched *M(3)B* homozygote showing abnormal midgut. Embryo slightly damaged in preparation.

histological examination. In the majority, in all six stocks, the midgut is usually unsegmented and crowded with yolk, the cephalopharyngeal apparatus may be undeveloped or only partially differentiated, and the nervous system is frequently disorganized or not condensed anteriorly (figs. 6, 8, 10, 11, 12). In some of these individuals, it would appear that coordinated development ceased around 12 to 14 hours of incubation. Although there are never very many of these abnormal types, they are most frequently encountered in *M(3)w* and *M(3)124*, less frequently found in *M(3)B* and *M(2)l²* and rarely found in *M(3)B²* and *M(1)o*. In addition, in *M(3)w* and *M(3)124*, three to five grossly aberrant embryos were found in which dorsal closure was incomplete or in which only two thirds of the embryo had developed with one end consisting of a cap of scattered yolk and a small amount of cytoplasm. Whether or not these rare types can be attributed to the homozygous presence of a Minute deficiency is not known.

Studies of sectioned embryos, fixed at various stages in development, reveal no differences between controls and Minute homozygotes until the time of yolk enclosure. At this stage in the lethal types, the midgut rudiments are slow to enclose the yolk and some embryos resemble the condition found in *M(2)l* (fig. 3). Occasionally, involution of the head and frontal sac formation is atypical and incomplete, but this occurrence is not common. It is probable that such abnormal embryos represent a portion of the unhatched, but developed eggs identified in egg counts.

In later stages, the only abnormality usually present involves the midgut. Although cardia and caecae form normally, the anterior and often the middle regions of the midintestine remain large, rounded and filled with yolk. This entire structure lengthens more slowly than is the case in controls and even in hatching stages, some undigested food is almost always present, a condition rarely encountered in wild type individuals (figs. 7, 9). In the majority of the sectioned homozygotes, the only basis for identification rested on the appearance of the midgut, for all other structures were morphologically normal.

Grossly, the homozygous Minute larvae of all stocks can be distinguished easily from controls. Such individuals are exceedingly small (around 1 mm) and do not increase appreciably in either length or width even though they may live for four or more days. Although normal feeding and crawling motions are carried out, such movements tend to be very sluggish. It has been found that lethal homozygotes survive longer when allowed to remain in the same dish with active growing sibs than when isolated to a separate container. It is thought that this greater longevity may possibly be attributed to the mechanical breaking up of the media by the larger larvae.

DISCUSSION

When the phenotypes of homozygotes of these seven Minutes, along with *M-4*, are compared with one another, certain striking similarities become apparent.

In all Minute types, the metabolism of yolk is impaired and this aberration is accompanied by abnormalities in midgut formation which range from extreme in *M-4*, to less severe in *M(2)l*. In the other Minute homozygotes, the only detectable deviation from the wild type lies in the slowness of midgut differentiation and yolk withdrawal. The cardia and caecae are absent altogether in *M-4* and poorly formed in *M(2)l*, although normal in the other Minutes of the series studied.

In the region of the foregut, head involution ceases at an early stage in *M-4* and proceeds further in *M(2)l*. In both cases, however, an abnormal cephalopharyngeal apparatus results. The foregut is normal in *M(1)o*, *M(2)l²* and in the third chromosome Minutes.

Other structures, such as the nervous system, can be arranged in the same sequence with respect to degree of development in different Minutes, *viz.* grossly abnormal in *M-4*, somewhat disorganized in *M(2)l* and comparable to controls in the case of the other Minutes. It should be noted that of those few homozygotes of the larval lethals which fail to hatch, a number exhibit the same pattern and type of aberrations which have been found in *M-4* and *M(2)l*.

Another similarity between these eight mutants is the time of appearance of the abnormalities. All anomalies can be traced to the same period in development, i.e., around 12 hours of incubation, a period just before and during which rapid changes involving different organ-systems occur. The abnormalities of the various Minutes are invariably found in one or more of the structures intimately concerned in this period of intense morphogenetic activity and can be recognized either in 12-hour stages or shortly thereafter.

The development of the phenotype of homozygous *M-4* has been discussed and interpreted in terms of a severe restriction in growth rate occurring at this critical period in development (FARNSWORTH 1957). The same hypothesis can be applied to the first, second and third chromosomal Minutes studied in the present work, with the modification that such a restriction, even though occurring at a similar time, must be less pronounced. Certainly differences in homozygous phenotype do occur between these eight Minutes, but they seem to be differences in degree, rather than in kind.

The pronounced mimicry in heterozygous phenotype displayed by the Minutes should also be considered. All Minutes are characterized by short bristles, dominance over wild type and by delayed development in larval stages. Even though the larval period is prolonged, heterozygous Minute larvae are smaller than their control sibs. This size difference also extends into the adult stage and has been shown by BREHME to be due to a decrease in cell size, not in cell number. A restriction in growth which affects the size of individual cells of many tissues must be due to an alteration of one or more general physiological processes common to most cells. If the presence of a given heterozygous deficiency results in delayed development and retarded growth, perhaps the homozygous phenotype is due to the same fundamental physiological aberration which becomes a completely limiting factor effective in earlier stages. It should be recalled that failure to grow is one of the outstanding characteristics of the larval lethals studied.

An explanation to account for the similarities in gene action on the part of these different loci can only be speculative at the present time. Further work to clarify the Minute phenotype on physiological grounds is being undertaken.

SUMMARY

1. The effects of seven different homozygous Minutes on the development of *Drosophila melanogaster* are described. The stocks studied include *M(1)o*, *M(2)l*, *M(2)l²*, *M(3)w*, *M(3)124*, *M(3)B* and *M(3)B²*.

2. Minute (2)l homozygotes die in the eggstage. Differences between controls and lethal homozygotes become apparent around twelve hours of development and consist of the following:

Fusion of the midgut rudiments is delayed and the wall of the midgut, when formed, is frequently incomplete, thus releasing yolk into body spaces. Lengthening and coiling of the gut proceeds slowly and even in late stages this structure remains large, sac-like and crowded with yolk, especially in more anterior portions. Cardia and caecae are poorly differentiated.

Abnormal head involution results in a small, aberrant cephalopharyngeal apparatus.

Some disorganization of the nervous system is usually present and condensation of the ventral ganglia is frequently incomplete.

3. Almost all homozygotes of the other six Minute stocks die in the larval stage and are practically indistinguishable from one another. The only morphological anomaly usually present appears around twelve hours of development and involves the midgut which is retarded in formation and differentiates more slowly than is the case in controls. Yolk frequently remains in the lumen of this structure even in hatching stages.

Hatched homozygous Minute larvae are approximately 1 mm in length and often live for several days. During this time, however, no appreciable growth occurs nor has any evidence of molting been observed.

In each of these six stocks, there are always a few homozygotes which fail to hatch. Such individuals are frequently characterized by abnormalities of the foregut, midgut and nervous system.

4. The similarities in the phenotypes of these seven Minute homozygotes are discussed.

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THE EFFECT OF LOZENGE PSEUDOALLELES ON EYE PIGMENTATION IN DROSOPHILA MELANOGASTER. I. BROWN PIGMENT AND PIGMENTATION IN LOZENGE MALES

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NUMEROUS investigations have been undertaken to determine the factors involved in producing the pleiotropic effects in the lozenge series of mutants. The recessive sex-linked mutants of this series affect in varying degrees eye color, eye shape and structure, viability and fertility, female reproductive structures, and the claws and pulvilli of the legs. GREEN and GREEN (1949) reported that the lozenge alleles were actually a group of pseudoalleles which could be assigned to three closely linked loci. They observed that the array of phenotypic effects of the mutants at the different loci were similar and that they were distributed at random when classified quantitatively on the basis of the amount of red pigment in the eyes.

If the lozenge gene controls one basic process during development, a single characteristic may secondarily produce others; whereas, if the gene produces several independent effects no single factor could be traced which would explain the multiple action of the gene. GRUNEBERG (1938, 1943), in a series of studies on mutants in mice, found that the multiple effects could be traced to a single biochemical effect early in development. He concluded that the pleiotropism of gene action is spurious rather than genuine, and that the multiple effects are the result of a single defect which arises during early development and secondarily produces the other anomalies. Some evidence for an interrelationship between the several pleiotropic effects in the lozenge series has been found. ANDERSON (1945) found that the reduced fertility of lozenge females was a secondary effect of the gene action on differentation in cells forming the female reproductive structures. OLIVER (1947), in his study of the interrelationship between eye color and facet arrangement in the lozenge series, suggested that the color and pigment distribution in the eyes are due in part to the relationship with facet arrangement and that both are secondary effects of the primary gene action on structure of the ommatidia. He observed, however, that there seems to exist some independence in the expressions of the lozenge gene on the quantities of red and brown pigment deposited in the eyes. In a study of the development of the eyes of some of the lozenge alleles, it was found (CLAYTON 1954b) that failure of the ommatidial cells to differentiate properly is the cause of facet irregularities in the adult eyes, but this developmental characteristic cannot completely explain variations in quantitative pigment differences.

The present paper is a study of the effect of ten members of the lozenge pseudoallelic series on the quantity and distribution of the eye pigments and the interrelationship between ommatidial abnormalities and eye color.

MATERIALS AND METHODS

The pigment analysis was made on the wild type, *Drosophila melanogaster* Stephenville, on the mutant brown (*bw*), and on ten members of the lozenge series. The lozenge mutants used were: lozenge (*lz*), lozenge 37 (lz^{37}), lozenge Bar-Stone (lz^{BS}), lozenge glossy (lz^{g}), lozenge 34k (lz^{34k}), lozenge y4 (lz^{y4}), lozenge 3 (lz^{3}), lozenge 36c (lz^{36c}), lozenge spectacle (lz^{s}), and lozenge spectacle-Bishop (lz^{sB}). Because of reduced viability and fertility, the lozenge cultures were maintained as balanced isogenic stocks, using the *ClB* inversion (OLIVER 1947). As a means of studying the effects of lozenge on brown pigment, isogenic stocks had been established in which each member of the lozenge series was homozygous for brown. All cultures were maintained at a constant temperature of 22°C on the standard banana-yeast laboratory food.

Since the age of the adult affects pigmentation, all observations were made on males collected from 48 to 72 hours after eclosion. Heads were dissected from the adults and placed into modified Carnoy's solution for thirty minutes. After fixation the material was cleared in xylol and imbedded in Tissuemat. Sections were cut at eight to ten microns. Stained sections were prepared by using Heidenhain's hematoxylin method with no counterstain; such slides were essential for the orientation of the pigment cells. Unstained slides were prepared by passing sectioned material rapidly through changes of xylol, absolute alcohol and xylol, followed by mounting in Permount. There was little or no loss of pigment with this technique and the unstained slides were used for the analysis of both brown and red pigment.

Whole mounts of adult eyes were prepared by fixing the heads in Cellosolve for fifteen minutes, clearing in xylol, and mounting in Clarite. Eyes prepared in this way retained their pigment and were useful in the examination of the external features of the eyes.

RESULTS

Pigmentation in the wild type eye

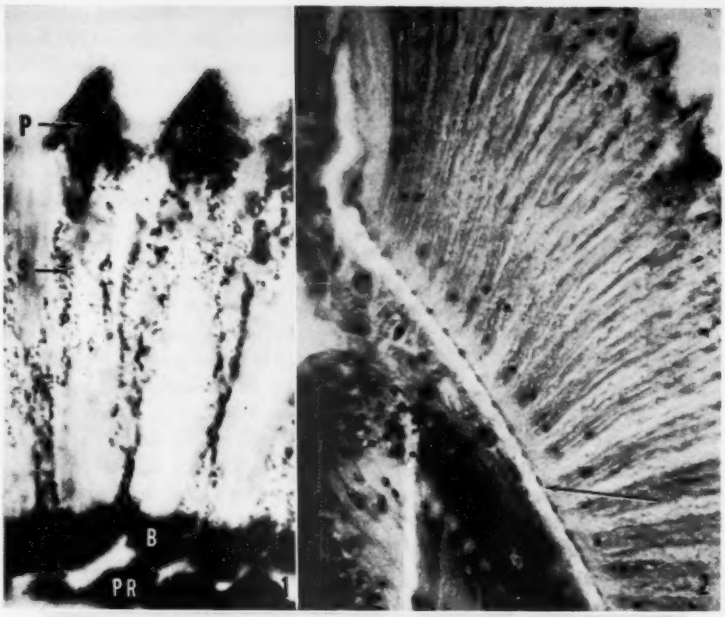
The normal color of the wild type eye is red, darkening slightly with age. Analysis of stained and unstained sections of normal eyes indicates that this color is the result of the presence of pigment granules distributed in four regions of each ommatidium (fig. 1). As described by NOLTE (1950), the pigment granules are of a similar type and size in each of the four pigmented regions and are equally distributed in number per unit volume of the cells. The four regions of pigment concentration described by NOLTE will be used as the basis for descriptions of pigment analysis in this study. A detailed description of the eye structure may be found in an earlier publication (CLAYTON 1952).

The outermost pigmented region is that composed of two primary pigment cells which surround the four pseudocone cells of each ommatidium. The granules of the primary pigment cells are red, giving an over-all impression of yellowish-red. The region of the secondary pigment cells forms the second concentration of pigment described by NOLTE. These cells are long and slender, extending from the basement membrane to the pseudocone region, with short processes apparently surrounding

the primary pigment cells. The outer, or distal, portion of these cells is similar in color to the first region of pigment concentration, giving an impression of yellowish-red. The granules of the inner, or basal, portion of the secondary pigment cells are a darker red, in aggregate appearing almost purple. The exact number of secondary pigment cells surrounding each ommatidium is not easily determined as the nuclei are not always located at the same level and the cell limits are often indistinct. The number of secondary cells for *D. melanogaster* has been described by JOHANNSEN (1924) and PILKINGTON (1941) as about twelve, as six by STRASBURGER (1935) and nine by NOLTE (1950). COCHRANE (1936) gave the number as twelve for *D. pseudoobscura*, and TATE (1948) described the secondary pigment cells as six to nine for Calliphora. Counts obtained from cross-sections of adult and pupal eyes in this study indicate that the figure given by NOLTE is probably accurate for *D. melanogaster*, although numbers from eight to ten were found occasionally.

The third region in which there is a concentration of pigment is that of the basal pigment cells. There have been a number of controversial reports on the existence of these cells. HERTWECK (1931) found no pigment cells near the basement membrane, and although JOHANNSEN (1924) described basal pigment cells with prominent nuclei, it is probable that these nuclei were actually those of the small eighth retinulae, which he did not report. CASTEEL (1929) described basal pigment cells in *D. melanogaster* as did PILKINGTON (1941). COCHRANE (1936) described enlarged proximal ends of the secondary pigment cells with a heavy concentration of pigment in *D. pseudoobscura*, but found no basal cells. NOLTE (1950), however, in both Zaprionus and Drosophila, clearly demonstrated the presence of the basal pigment cells encircling the bases of the ommatidia, with their nuclei lying flat against the basement membrane. He found that these nuclei were present in groups of nine around each ommatidium, although his counts varied at times from eight to ten, a condition similar to that of the secondary pigment cells. Although these cells are often difficult to observe in the adult eyes, they may be seen quite clearly in late pupal stages. Figure 2 is the photograph of the eye of a recently emerged *Drosophila virilis* male and the basal pigment cell nuclei can be seen lying flat against the basal membrane and corresponding in location to the basal region of each ommatidium. A similar orientation was seen in the eyes of *Drosophila melanogaster* pupae. The nuclei of these cells are characterized by the separation of the nuclear material into two masses. NOLTE suggested, as a probable orientation of the basal pigment cells, the presence of sheathlike processes around the tips of the secondary pigment cells. Abnormalities in the organization of cells in certain eye mutants could result from the failure of these sheaths to remain interlocked. The granules of the basal pigment cells are a dark red, giving an impression of purplish red when in aggregate.

The fourth concentration of pigment lies beneath the basement membrane in the postretinal region. POWER (1943), in describing the outer optic ganglion of the brain, listed three layers: the outer fenestrated zone immediately under the basement membrane, a more proximal layer of monopolar neurons, and an innermost layer, the external glomerulus. The pigment is located in the fenestrated zone, or postretinal regions. CASTEEL (1929) and HERTWECK (1931) mentioned the presence of pigment beneath the basal membrane in *D. melanogaster* and COCHRANE (1936) found this



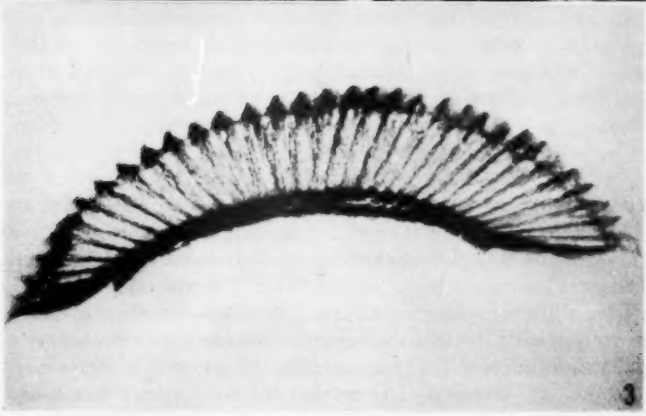


FIGURE 1.—Ommatidium of normal eye showing distribution of pigment granules in four regions: (B) basal pigment cell, (P) primary pigment cell, (PR) postretinal pigment cell, and (S) secondary pigment cell. Unstained, ×900.

FIGURE 2.—Compound eye of *Drosophila virilis*, young adult. The arrow indicates the basement membrane and the row of basal cell nuclei. Pigment has been removed from the ommatidia. Iron hematoxylin, ×900.

FIGURE 3.—Pattern of pigmentation in normal eye. Unstained, ×200.

pigmented region in eyes of *D. pseudoobscura*. However, these three authors presumed this pigment to be present in processes of the secondary pigment cells extending through the basement membrane. This study, and the one made by NOLTE (1950), indicate that this concentration is probably the result of distinct pigment cells in the postretinal region. The pigment masses are usually separated from the basement membrane and the monopolar neurons by narrow spaces through which pass the nerve fibers of the retinulae. Also, these pigmented masses appear to correspond in number and location to the ommatidia above them. The postretinal pigment cells are shown in figure 2 as distinct cells oriented below the basement membrane and aligned with the ommatidia. Although this question cannot be completely answered, the results of this study indicate that distinct pigment cells probably do exist in this region. The over-all impression of granule color in the postretina is similar to that of the basal pigment region, appearing as a dark purplish-red color. The photograph of an unstained section of wild type *D. melanogaster* eye (figure 3) indicates the general pattern of pigment deposition in these four pigment cell regions.

Pigmentation in the lozenge series

The lozenge alleles vary considerably in the amounts of red and brown pigments present in the adult eyes, most of the alleles affecting both quantity and distribution of the pigments. OLIVER (1947) described the eye color seriation for the same alleles used in this study and reported that the eye color series did not correspond with that for facet irregularity. One group of alleles may be graded from normal eye color to dark sepia while the others have a yellowish brown color with a webbed arrangement of red and a darker rim of color. The facet irregularities may be graded from the nearly normal condition of lz^{37}, in which only a few facets are involved, to those mutants with no normal facets. Detailed descriptions of the structural abnormalities and development of the lozenge eyes may be found in earlier publications (CLAYTON 1952, 1954b).

Lozenge-37

The eyes of lz^{37} males have only small areas of irregular facets which are usually found in the posterior region of the eye. The ommatidial abnormalities correspond in general to the region of facet fusion although some abnormalities appear in the region of the basement membrane below normal facets. The eye color is normal and the over-all color of the pigment granules cannot be distinguished from wild type. The only effect of this mutant on the eye pigment is an irregularity in distribution due to structural abnormalities of the pigment cells. The pigment in the primary cells is distributed normally except in those regions of fused facets. Where fusion has occurred the primary pigment cells are distorted in shape but the amount of pigment appears to be unaltered. The distal portions of the secondary pigment cells are normal in number and arrangement. Serial cross-sections through these cells show that, although the distal portions are normal, the proximal portions may be distorted in those regions of structural abnormality. In areas where the basement membrane is disrupted by abnormal retinulae the basal pigment cells and proximal portions of secondary pigment cells are distorted and irregular in shape, thus causing irregulari-

ties in the distribution of pigment in these regions. Occasionally the abnormal arrangement of cells in the basal region is such that no pigment is present in the region surrounding displaced retinulae. The most severely affected concentration of pigment is the region below the basement membrane, that of the postretinal pigment. The normal arrangement of the postretinal pigment is disrupted in those regions which have abnormal retinulae below the basement membrane and heavy streaks of pigment are found surrounding these abnormal cells. In the normal areas, in which the irregularities are restricted to the postretinal region, the abnormalities of pigment distribution are also restricted to this area. In normal parts of the eye no abnormalities in pigment concentration or distribution could be distinguished.

Lozenge

The eyes of lozenge are rougher than normal, with patches of fused and abnormal facets. The eye color is normal except in regions of facet abnormality. In fused areas the color may appear as a brighter red or as small dark spots where erupted facets occur. The color in unstained sections is like wild type, with pigment irregularities restricted to regions of abnormal cell arrangement. The structural abnormalities are more severe than in lz^{37} and the irregularities in pigment distribution show a corresponding change. The dark spots seen on the eye surface appear in sections as brownish areas in the cornea. These areas appear to be the result of "eruption" facets formed during pupal development. The distribution of pigment in the primary pigment cells is normal except in areas of fused facets. These pigment cells in such regions usually fail to develop pseudocones and lie flat beneath the cornea. This altered relationship of the cornea and the shallow primary pigment cells may account for the brighter red seen in regions of fusion. In the areas of facet abnormality the pigment is dense and distributed irregularly in all surrounding pigment cells. In the basal and postretinal regions, the irregularities are greatest. The disruption of these areas by the postretinal layer of retinulae causes heavy concentrations of pigment in some areas and complete absence in others. The postretinal pigment frequently forms long heavy streaks around the abnormal retinulae but does not always accompany these abnormal cells. In normal regions of the eye, the concentration and distribution of pigment granules are normal.

Lozenge Bar-Stone

The eyes of lozenge Bar-Stone have fused facets scattered over the surface of the eye. The size and number of these fused regions is variable but in the larger areas of fusion the facets form warty spots and appear shiny. The eye color varies with the severity of the irregularities, the fused areas frequently appearing as shiny red blisters. In sections these fused areas can be located above the severest abnormalities in cellular arrangement and pigment distribution. All areas of pigment concentration are abnormal in lz^{BS} eyes (fig. 4). The primary pigment cells are present in a layer beneath the fused facets and the pigment concentration is quite dense. The retinulae are distorted and the secondary pigment cells surrounding these cells are disarranged. The basal pigment cells and postretinal cells are disrupted by the second layer of retinulae, located beneath the basement membrane. The elongation of these retinulae

during development results in the disruption of the pigment in the basal and postretinal cells. Postretinal pigment frequently surrounds the retinulae in this second layer, though not as regularly as in lz^{37} or lz. The distribution is quite irregular, the pigment appearing as streaks adjacent to the abnormal retinulae or being almost completely absent. The general color of the eye in unstained preparations is similar to wild type in normal regions but is quite variable in abnormal areas, varying from a bright red to a very dark red.

Glossy

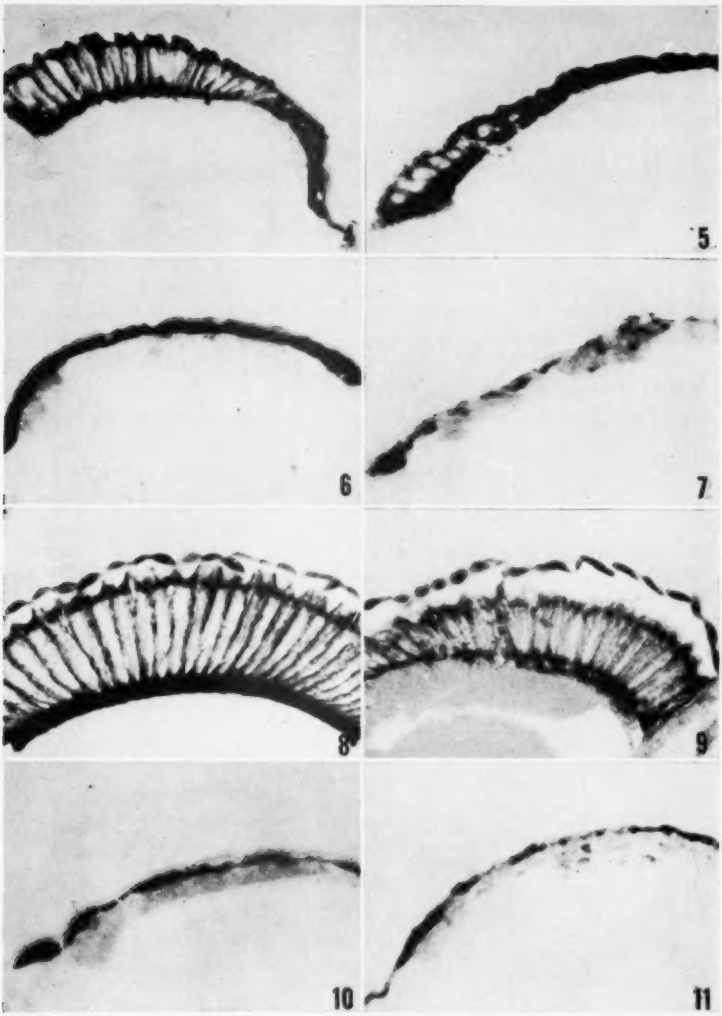
The regions of fusion are much more extensive in lz^{g} than in lz or lz^{BS}, giving the eye an over-all glossy appearance. The eye color is not normal, but appears as a dark blood-red with bright red shiny spots in some abnormal areas. The unstained sections of glossy show these same variations in eye color. The normal and abnormal areas are very dark red except in scattered spots where the structural abnormalities are very severe. In these extreme regions a solid layer of red pigment is present beneath the flattened cornea. The irregularity in pigmentation in different regions of the eye is shown in the photograph of an unstained section of the lz^{g} eye (fig. 5). The color in the pigment cells is quite variable; the distal portions of the secondary pigment cells appear almost orange in color while the proximal portions are dark red. Postretinal retinulae are not accompanied by pigment except in occasional regions where a few scattered granules may be seen. In regions where normal ommatidia occur the basal and postretinal pigment concentrations are thicker than in wild type and the pigment granules are very dense, giving a dark red color.

Lozenge-34

The eyes of lz^{34} have large fused areas with occasional normal facets. This mutant is the most severe among the alleles possessing some normal ommatidia. OLIVER (1947) described the eye color as a dark maroon-red with small yellowish spots surrounded by heavy aggregates of red color. The color in sections is a dark red with variations in this color in regions of greatest structural irregularity. The cornea in some facetless regions forms depressed cuplike areas surrounded by highly distorted pigment cells and retinulae. Pigment is almost completely lacking in the regions just below these depressions but a layer of primary pigment cells with a heavy red pigment deposit occurs around the depressed cornea. The structural irregularities of the ommatidial cells in lz^{34} are so severe that few normal regions could be observed. The postretinal layer of retinulae is highly developed and deposits of pigment in this region are scattered, sometimes accompanying the retinulae, but more frequently occurring as patches of very dark pigment. In those surface areas where there is no indication of normal facets and the cornea is a flattened sheet the pigment occurs as a thick, very dark layer below the cornea.

Other alleles

The structural abnormalities of the remaining alleles (lz^{v4}, lz^{3}, lz^{s}, lz^{sB}, and lz^{36}) are quite similar and accurate differentiation could not be made on the basis of pigment distribution or ommatidial abnormalities. However, differences in the degree



FIGURES 4–11.—Photographs of compound eyes of the mutant brown and several lozenge pseudoalleles. Figures 4 through 7 show the distribution and quantity of both the red and brown pigments. The normal distribution of brown pigment, Figure 8, may be compared with variations in three lozenge pseudoalleles homozygous for brown, Figures 9 through 11. Figure 4. $lz^{B\,S}$; Figure 5. lz^{2}; Figure 6. lz^{3}; Figure 7. $lz^{2\,B}$; Figure 8. Brown; Figure 9. $lz^{B\,S}$ *bw*; Figure 10. lz^{3} *bw*; Figure 11. $lz^{2\,B}$ *bw*.

of pigmentation do exist. Two of these mutants, lz^{v4} and lz^{3}, could not be distinguished in the histological study (CLAYTON 1952); yet they are quite different in their effects on pigment deposition. The eyes of lz^{v4} are dark red with a heavy band of pigment at the margin and uneven streaks toward the center. This pigment appears in sections as a layer of dark red granules beneath the flattened cornea, with a thicker concentration at the rim. The concentration of pigment around the abnormal retinulae is sparse, appearing only occasionally as thin streaks of pigment around the retinulae. The pits and eruptions of the cornea are accompanied by a decreased amount of pigment so that the color in such regions is a bright red.

The eyes of lz^{3} are dilute in color, appearing more brown than red. Around the margin there is a heavy concentration of reddish-brown pigment. With different individuals the color in unstained sections varies from a yellowish brown to a reddish brown. The distribution of the pigment is the same as in lz^{v4}, with a layer of pigment below the cornea and a heavier concentration at the rim (fig. 6). The thickness of the pigment layer toward the center of the eye is variable and the color of a particular region depends upon this density. Some pigment is present with the retinulae but this pigment deposition does not occur regularly.

The eyes of lz^{s}, lz^{sB}, and lz^{36} are yellowish brown with a fine stippling of small red-brown spots and a rim of a reddish-brown color. The color of the eyes seems to be variable, appearing in sections as shades from a pale yellowish brown to a deep reddish-brown. The pigment layer beneath the cornea is not as thick in these mutants as in lz^{3} or lz^{v4}. Some regions lack pigment completely but are surrounded by patches of dull brown granules. In the rim, where a large concentration of pigment occurs, the color is reddish-brown. Only occasional streaks of pigment occur around the highly distorted retinulae. The pattern of pigmentation in this group of mutants can be seen in the photograph of lz^{sB} (fig. 7).

Effect of lozenge on brown pigment

The eye color of the brown mutant is tan or light brown on emergence of the adult, darkening with age to garnet. EPHRUSSI and HEROLD (1944) reported that the recessive gene brown (*bw*) removes or suppresses all of the red pigment and also decreases the amount of brown pigment. NOLTE (1950) found that at emergence the color of all granules is the same, but after one day two types of brown can be observed. In the primary and basal pigment cells the granules in aggregate appear yellowish brown, whereas the granules of the secondary and postretinal pigment regions are darker, a purplish brown. The postretinal region appears to be slightly narrower than in wild type. The pigment distribution in the eyes of brown is shown in figure 8.

The phenotypic effect of lz^{37} on pigment with *bw* present is limited to the regions of structural abnormalities. In normal regions the amount and distribution of the pigment are normal and in the abnormal regions the variations in pigment distribution are similar to those previously described for this mutant. With *bw*, however, pigmentation in the basal and postretinal regions is more irregular than in the previously described condition. Around abnormal retinulae in the postretina the pigment granules are sparse and may be completely absent in some irregular regions. The

basal pigment is scattered where the basement membrane is disrupted and heavy streaks of pigment from the secondary pigment cells surround abnormal retinulae. The brown pigment granules appear to be normally distributed in the distal portions of secondary pigment cells and in primary pigment cells above disrupted basal and postretinal pigment cells. The eye color in abnormal regions varies from normal to a light brown.

The eyes of *lz bw* are wine-red in color and appear darker than brown. The deeper color results from a dense concentration of granules in the primary pigment cells in both normal and abnormal areas. In *bw*, the outer pigment is yellowish-brown, whereas, in *lz bw* the outer deposit of pigment is dark red. Irregularities in the distribution of the pigment granules are similar to those described previously. The absence or abnormality in shape of the peudocone cups alters the relationship of the primary pigment to the surface and this orientation, together with increased pigment deposition, would account for the eye color which is darker than brown. Disruption of pigment cells is most severe in the region of the basement membrane and the distribution of the brown pigment is irregular wherever structural abnormalities occur.

The Bar-Stone allele has a heavy concentration of brown granules in the marginal regions, in aggregate appearing reddish-brown. Except in this rim the color is yellowish or dull brown. The amount of pigment present in normal regions is decreased in the primary cells and in the distal portions of the secondary cells, but it appears to be normal in the proximal portions of the secondary cells, in the basal cells and the postretinal cells. In abnormal areas reduction in the amount of pigmentation is present in all pigment cells; wherever structural abnormalities occur, the distribution of the pigment is irregular and in very severe regions may be completely lacking (fig. 9).

The eye color with glossy appears brown, lacking the reddish color of lz^{gs} except in some very abnormal regions. In areas where a dense concentration of pigment granules occurs the color is reddish-brown. The pigment cells of normal ommatidia are similar to those of the brown mutant but in abnormal regions pigment distribution is irregular in all pigment cells. The amount of pigment in structurally abnormal cells varies, particularly in distal portions of the ommatidia. Some primary pigment cells appear to have a normal amount of pigment, whereas there is a reduction in other cells. Less pigment than in *bw* is characteristic of the secondary, basal and postretinal pigment cells of abnormal regions. There is less brown pigment deposited in the eyes of lz^{34} than in any of the previous mutants. Large masses of pigment granules concentrated below the cornea or near the basal membrane are dull brown in color, whereas other areas are colorless. There is very little pigment present in the postretinal area, but occasional spots or streaks of pigment can be seen around abnormal retinulae.

The brown pigment in lz^3 is concentrated in a heavy layer beneath the cornea with a thicker concentration at the margins (fig. 10). In the slides analyzed the marginal rim appeared to be thicker in the posterior portion of the eye, with a much narrower rim at the anterior margin. Pigment is present around the distorted retinulae to a limited degree; however, the pigment cells are so severely distorted that the distribution is very irregular.

In both lz^{sB} and lz^{36} the brown pigment distribution is similar to the pattern in these mutants when both red and brown pigments are present. This can be seen by comparing the photographs of lz^{sB} and lz^{sB} *bw* (fig. 7 and 11). The only distinction that could be made was that the color in both lz^{sB} and lz^{36} was light brown except in the margins where the color varied from dull brown to purplish brown. The reddish brown stippling is not present with *bw* and the amount of pigment in the rim is not as great as when red pigment is also present. No study was made of either lz^{w4} *bw* or lz^{s} *bw*.

DISCUSSION

In their influence upon the structure and pigmentation of the compound eyes the lozenge alleles vary considerably in the degree of their phenotypic effect. The histological study of the eye structure (CLAYTON 1952) indicates that abnormalities in facet arrangement may be correlated with the underlying anomalies in structure of the ommatidial cells. Seriation of these structural defects corresponds to the series developed by OLIVER (1947) for facet arrangement. OLIVER also placed the alleles in series based upon the amount of red pigment and brown pigment produced by each allele. He found that the seriation used for facet irregularity could not be used for eye color when both pigments were considered. One set of alleles could be seriated from normal to increasing darkness, whereas the color was greatly reduced in others. However, if the red and brown pigments were considered separately linear series based upon the quantity of red or brown pigment could be made.

The color in unstained sections of the lozenge alleles form series identical to those given by OLIVER. When both red and brown pigments are present the mutants can be placed into two groups. Ranging from normal coloration to a color darker than wild type are lz^{37}, *lz*, lz^{BS}, lz^{g}, lz^{34} and lz^{w4}. The remaining mutants, lz^{3}, lz^{s}, lz^{sB}, and lz^{36}, form a second group in which the red pigment is greatly reduced and the eyes have a brown appearance, although some reduction in brown pigment also occurs. The amount of pigment present in the different regions of pigment concentration varies with the allele. The quantity of pigment in lz^{37} and *lz* appears normal for each pigmented region, with the distal pigmented areas appearing yellowish-red and the proximal regions appearing as a deep red. In the remaining mutants with a dark eye color, the irregularities in the distribution of the pigments are such that it is difficult, from examination of sections, to compare the total amount of pigment with that of wild type.

In lz^{37} and *lz* the distribution is almost normal, but in the other alleles of this group the distribution is irregular. In lz^{BS}, lz^{g} and lz^{34}, the general color is similar to wild type in those regions unaffected structurally but in the abnormal areas the color varies from bright red to deep wine red. This variation, when present in the primary pigment cells, may account for the over-all dark red eye color since normally this outer portion of the eye is yellowish red. This agrees with OLIVER'S observations (1947), since increasing facet irregularity is accompanied by an increase in the intensity of the eye color. The darkest eye color is found in lz^{w4}, which also shows the greatest abnormality in pigment distribution. The pigment in this allele is heavily concentrated in a layer just beneath the cornea.

In lz^3, lz^s, lz^{sB}, and lz^{36}, the extreme reduction in the quantity of red pigment, as well as a less severe reduction in brown, makes the eyes appear a dilute brown in color. Each of these alleles possesses a reddish-brown marginal rim with a fine stippling of pigment over the eye. The stippling effect is the result of small spots of pigment beneath the flattened cornea. The patches of pigment are variable in color, most frequently appearing as a dull brown. As suggested by OLIVER (1947) the overall eye color is probably the result of a general dilution of color resulting from the rather uniform scattering of the eye pigment.

When the red and brown pigments are analyzed separately the effect of lozenge on the quantity of each pigment can be more easily determined, and a linear series of the alleles may be developed for differences in the quantity of pigment produced for red or brown eye color. The relative order of the effect of the alleles when vermilion is present in the genotype has been given by OLIVER (1947) as follows: lz^{37}, lz^g, lz and lz^{BS}, lz^{34}, lz^{v4}, lz^3, with lz^s and lz^{36} containing the smallest amount of red pigment. This series corresponds to that given by GREEN (1948) based on the quantitative anlysis of red pigment extracted from lozenge mutants when vermilion was present. The histological analysis of the lozenge series in stocks homozygous for the mutants scarlet or vermilion is in progress and will be reported later. OLIVER'S seriation of the lozenge mutants homozygous for brown differs from that given for the red pigment. In this series, lozenge (lz) has the greatest amount of pigment, followed by lz^{37}, lz^g, lz^{BS}, lz^{34}, lz^3, lz^{v4}, lz^s and lz^{sB}, and lz^{36} in the order of decreasing amounts of brown pigment.

Although there is a reduction in the quantity of red and brown pigments produced in the eyes of all of the lozenge mutants except lz^{37} and lz, the eye color appears darker than wild type in several members of the series. Contrasts of the red and brown pigments which could intensify the eye color, together with the altered distribution of the pigments, may account for the increasing darkness of color in some of the mutants. The distribution of pigment in different regions of the eye is directly related to the irregularities in arrangement and structure of the ommatidia. Increased abnormalities in the arrangement of the ommatidia result in corresponding displacement of the accompanying pigment cells.

The brown pigment in lz^{37} appears normal in quantity with the yellowish brown color in the outer pigment cells and the dark reddish-brown in the basal and postretinal concentrations. The amount of brown pigment in lz was reported by OLIVER (1947) to be greater than that of wild type or lz^{37}; the study of unstained sections confirms this observation. The pigment distribution is regular, but the amount of pigment is greater, particularly in the primary pigment cells and the distal portions of the secondary pigment cells. The granules are a uniform wine-red color throughout the eye instead of appearing yellowish-brown in distal regions where normally the brown pigment is less concentrated. In the remaining alleles the amount of brown pigment is reduced and a linear series, which corresponds closely with the series based on ommatidial abnormalities can be arranged. The only exception is that lz^g, structurally more severe than lz^{BS}, has slightly more brown pigment than Bar-Stone.

The relationship of pigmentation in the lozenge series of mutants to the abnormalities in cellular differentiation is clear as shown by the histological studies of a number

of these alleles. The crossover tests carried out by GREEN and GREEN (1949) indicated that the lozenge mutants are pseudoalleles at three closely linked loci. The original tests placed lz^{BS}, lz^{46} and lz^{g} in that order from left to right on the X chromosome. Their later tests (unpublished, cited in CLAYTON 1954a) have made it possible to assign other lozenge mutants to these three loci: allelic to lz^{BS} are lz^{37}, lz^{s}, lz^{36} and lz^{34}; allelic to lz^{46} is lz; allelic to lz^{g} are lz^{3} and lz^{v4}. The classification of these peudoalleles on the basis of quantitative differences in red pigment (GREEN and GREEN 1949) and on the basis of eye structure in lozenge compounds (CLAYTON 1954a) is a random distribution. No evidence was found in either of these studies that would indicate a direct correlation between the locus of the allele and its phenotype. Interactions of the mutants with either true alleles or pseudoalleles could not be explained solely on the basis of the locus, or loci, involved in the heterozygous combinations.

SUMMARY

The effect of ten members of the lozenge series on the quantity and distribution of the eye pigments is described and the pigmentation in males homozygous for the mutant brown is analyzed.

When both red and brown pigments are present, the lozenge alleles may be classified into two groups, the first consisting of those mutants which range from normal coloration to a color darker than wild type: lz^{37}, lz, lz^{BS}, lz^{g}, lz^{34}, and lz^{v4}. The second group is composed of those alleles with eyes brownish in appearance: lz^{3}, lz^{s}, lz^{sB}, and lz^{36}. When the brown pigment is analyzed separately a single series may be arranged, with the exception of lz which seems to be darker than normal, in the order of decreasing amounts of brown pigment. This seriation corresponds to a series based upon increasing severity of ommatidial abnormalities.

The irregularities in the pigmentation of the eyes of the lozenge alleles may be partially explained on the basis of abnormalities in the structure and size of the pigment cells but it is suggested that some independent action of the gene results in variations in the quantities of red and brown pigment present in the alleles.

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CYTOLOGICAL ANALYSIS OF X-RAY-INDUCED MUTATIONS AT EIGHT SPECIFIC LOCI IN THE THIRD CHROMOSOME OF DROSOPHILA MELANOGASTER[1]

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The application of cytogenetic techniques to studies of radiation effects gives a measure of the physical damage to the chromosomes. Such qualitative and quantitative measures of the chromosomal damage and correlation of these results with genetic tests are necessary for the detection and separation of mutational pattern differences in various types of germ cells or mutational pattern differences as a result of various types of radiation. It is with this combination of cytological and genetic techniques that we come closest to determining the exact nature and extent of heritable damage to the organism.

Numerous investigations on the induction of lethal and visible mutations have been carried out; however, in many instances, cytological analysis of these mutations to determine the chromosomal changes associated with the genetic changes has not been practical. Much of the material was not suitable for a detailed cytological analysis, but with the discovery that the salivary gland chromosomes of the dipterous insects could be used for cytogenetic analysis (PAINTER 1933), *Drosophila melanogaster* became the ideal organism for such studies.

The first application of combined genetic and cytological tests to a group of X-ray-induced specific autosomal mutations was by PAINTER and PATTERSON (1935) who used X-ray-induced mutations at eight specific loci in the autosomal third chromosome of *D. melanogaster*. (See also ALEXANDER 1954.) The present investigation also combines genetic and cytological analyses. The X-ray-induced genetic damage was recovered as visible mutations at eight specific loci on the third chromosome of *D. melanogaster*, seven of which were used by PAINTER and PATTERSON. These mutants were analyzed genetically for viability and cytologically for the presence of chromosomal aberrations. In addition to the determination of the types and frequencies of the chromosomal changes, the types and frequencies of changes induced in the ma-

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ture sperm from irradiated 7-day-old males are compared to the changes that were induced in spermatogonial cells from treated larvae.

Using the various deficiencies, inversions and translocations analyzed in this investigation, we were able to confirm the previously reported locations of some of the loci, and in some instances to localize certain of the other genes more accurately.

MATERIALS AND METHODS

X-ray-induced visible mutants involving eight specific loci, roughoid (*ru*), hairy (*h*), thread (*th*), scarlet (*st*), pink (p^p), curled (*cu*), stripe (*sr*), and ebony (e^s) on the third chromosome of *Drosophila melanogaster*, were studied cytologically and genetically. These mutants were obtained from mature sperm treated with 3000r of X-irradiation and spermatogonia treated with 900r of X-irradiation. Males that had been treated either as adults (for mature sperm) or as larvae (for spermatogonia) were mated to homozygous marker females. All F_1 variants that resembled any of the eight mutant markers were tested by crossing over in order to limit the variants to genetic mutations that involved the regions of the eight marker mutants. Viability tests for induced mutations were based on the ratios obtained from crosses of individuals heterozygous for the induced mutant and a test marker, either p^p or e^s. The occurrence or modification of the ratio of 1 test marker:2 normal:1 induced mutant determined the classification of the induced mutant into one of several categories. Induced mutants that occurred in an equal number with the test marker were classified as viable mutants, and those which occurred less than one half as often as the expected frequency were classified as semilethal. The absence of the third class (induced mutant class) indicated that the mutant was lethal in the homozygous condition. Some *sr* mutants were tested by this method, but since none of the induced mutants could be recovered homozygous, all were retested with the balancer chromosome, *In(3R)C, Sb e l (3)e*, which contains ebony to allow sharper classification of the stripe phenotype. Complete details of the genetic analyses of these mutants have been reported by ALEXANDER (1954).

Cytological analyses of viable mutations were made by mating homozygous mutant flies to the Oregon-R strain of *D. melanogaster* and examining the salivary gland chromosomes of the F_1 larvae. Lethal and semilethal mutants involving seven of the eight loci were balanced against the *C(3)X* chromosome and the stripe mutants were balanced against the *In(3R)C* chromosome. Males that were heterozygous for the induced mutant and a balancer chromosome were mated to Oregon-R females and the F_1 larvae studied cytologically.

RESULTS

Twenty-six of the fifty-six mutations that were induced in mature sperm were associated with cytologically detectable chromosomal aberrations. The descriptions of the various aberrations analyzed in this investigation are given in table 1. All locations are given according to C. B. BRIDGES' chromosome map (1935) of *D. melanogaster*.

A summary of the cytological positions of the eight loci as determined by the 26 chromosomal aberrations is given in table 2, which includes the number and types

TABLE 1

Rearrangements occurring with mutations

Gene	Number and type	Left break	Right break	Added information and remarks
roughoid	100.392 Deficiency	Left of 61F1	Between 62A6 and B1	
	100.393 Deficiency	Between 61F2 and 3	Between 62A4 and 6	
	300.234 Deficiency	Left of 61F1	Between 62A2 and 3	
hairy	100.12 Inversion	Between 61A2 and 3	Left of 66E1	
	100.239 Inversion	Between 66D3 and 4	80C	
	100.271 Translocation (2–3)	Between 66D5 and E1		Other break, heterochromatic region of 2R
	100.390 Deficiency	Between 66D1 and 2	Between 66D5 and E1	
thread	100.293 Translocation (transposition)	Between 72A2 and B2		Segment from 76A4-B1 through 79A1-B1 is deleted and inserted into 72A in inverted sequence.
thread scarlet	100.105 Deficiency	Between 72A2 and B1	Between 73A4 and 5	
scarlet	100.62 Deficiency	Between 73A2 and 3	Between 73A5 and B1	
	100.126 Translocation (Y-3)	Between 73A2 and 3		Y-3 Translocation
	100.171 Deficiency	Between 72E2 and 3	Between 74C2 and 3	
	100.200 Deficiency	Between 72E2 and 3	Between 73A5 and B1	
	100.359 Translocation (2–3)	Between 73A2 and 3		Second chromosome is broken between 21C3 and 5; one break in third chromosome is between 73A2 and 3 (between doublets) and second between 98F2 and 4. The distal portion of 2L is attached to the proximal portion of 3L, the distal portion of 3R to the proximal portion of 2L, and the distal portion of 3L to the proximal portion of 3R.

TABLE 1—*Continued*

Gene	Number and type	Left break	Right break	Added information and remarks
pink (*peach*)	100.48 Inversion	In heterochromatic region of centromere	Between 85A3-B1 (to the right of dark bands in 85A) and B1	
	100.88 Inversion	In heterochromatin of 3	Left of 94E1	This inversion is probably not associated with the *pink* region.
	100.290 Inversion	Right of 85B2 (a very light band)	Right of 85D10 (the most prominent band in D)	
curled	100.69 Translocation (X-3)	Between 88A4 and B1		X chromosome broken between 6B1 and C1.
stripe	100.312 Translocation (2–3)	Between 90D2 and E1		Heterochromatic region of 2.
	100.394 Deficiency	Between 90C1 and 4	Between 90F3 and 7	
	100.23 Translocation (Y-3)	Between 90E2 and 3		Y-3 Translocation
	300.24 Deficiency	Between 90C1 and 2	Between 91A2 and 4	
	300.101 Deficiency	Between 90D2 and 4	Between 91A4 and 5	
	300.240 Translocation (transposition)	89E	92A	The segment extending from 89E to 92A was deleted and inserted in region 75C in inverted order.
ebony (*sooty*)	100.172 Deficiency	Between 93B6 and 8	Between 93F7 and 94A1	Left break is to the right of first three doublets.
	100.256 Deficiency	Left of 93B1	Between 93F2 and 5	
	100.265 Inversion	Between 93B4 and 5	95E	Left break is just to the right of the two darkly staining doublets.
	100.307 Inversion	Between 62E2 and 3	Between 64C2 and 3	This rearrangement is independent of the mutation at the *ebony* locus.
	300.96 Inversion	Between 89F2 and 90A1	Between 99B1 and 2 (next to the doublet)	This rearrangement is probably independent of the mutation.

TABLE 2

Cytological positions of the eight loci of "res" *on the third chromosome of* Drosophila melanogaster

Locus	Cytological Position		Number and types of aberrations from present investigation
	Bridges, 1935	Present Investigation	
roughoid	61F	61F or 62A	3 deficiencies
hairy	66D	66D	2 inversions 1 translocation 1 deficiency
thread	72A-D	72A-72B1	1 deficiency 1 transposition
scarlet	75B-76D	Dark doublet 73A3-4 or band 73A5	4 deficiencies 2 translocations
pink	84F2-85C-D	85A3-85D10	2 inversions
curled	86E2-87D-E	88A4-B1	1 translocation
stripe	89A-89B	90C1-90F7	3 deficiencies 3 translocations
ebony	91A	93B1-93F5	2 deficiencies 1 inversion

of aberrations used to determine each locus and the positions reported by BRIDGES (1941a, b). The *ru*, *h*, *th*, and p^p loci all fall within the regions indicated by previous investigators. Our data indicate a different location for each of four of the genes, *st*, *cu*, *sr*, and e^s from that previously reported. In each case, the new location was always somewhat farther from the centromere region than that reported by BRIDGES.

The genetic and cytological comparisons of the mutants induced in mature sperm and spermatogonia are presented in table 3. Each mutant was placed in one of four cytological categories: no aberration, deficiency, inversion, or translocation. Each category was subdivided on the basis of the genetic viability of the mutant. The viable class includes mutants that survive in a homozygous condition with no apparent reduction in viability while the semilethal classification indicates that not more than half of the expected number of homozygous mutant individuals survived. The lethal class includes those in which no homozygotes survived. The totals and percentages of mutations in the various categories are given in the extreme right columns of the table. Mutations from irradiated sperm and spermatogonia were totaled separately and the totals for the various loci are given at the bottom of the table. One deletion, involving both the *th* and *st* loci, is recorded in both columns but is counted only once in determining the total number of mutations. In three cases inversions were present that obviously did not involve the region of the mutated gene. One inversion was present in the chromosome with a p^p mutation (100.88) and in two instances inversions were present with e^s mutants (100.307 and 300.86); these mutants were placed in the "no aberration" category.

Eight mutations which were induced in spermatogonia were analyzed. All genetic categories were represented, but no chromosomal aberration was detected. In the test of the mutants induced in mature sperm, about one half were associated with chromosomal aberrations and the remainder with no detectable changes. In the "no aberra-

TABLE 3

Results of cytological and genetical analyses of X-ray-induced mutations

Cytological Analysis	Loci								Total			
									Sperm		Spermatogonia	
	ru	*h*	*th*	*st*	*p*[p]	*cu*	*sr*	*e*[s]	Number	%	Number	%
No aberration	1	(2)	2	1	6[b]	7(2)	3(2)	10[c](2)	30	53.6	8	100
Viable	1			1	3	5(2)		6(2)	16		4	
Semilethal		(1)			1	1			2		1	
Lethal		(1)	2		2	1	3(2)	4	12		3	
Deficiency	3	1	1[a]	4[a]			3	2	13	23.2		
Viable												
Semilethal												
Lethal	3	1	1[a]	4[a]			3	2	13			
Inversion		2			2			1	5	8.9		
Viable		1			2			1	4			
Semilethal		1							1			
Lethal												
Translocation		1	1[d]	2		1	3[d]		8	14.3		
Viable												
Semilethal												
Lethal		1	1	1			2		5			
Total tested	4	4(2)	4[a]	7[a]	8	8(2)	9(2)	13(2)	56		8	
Viable	1	1		1	5	5(2)		7(2)	20		4	
Semilethal		1(1)			1	1			3		1	
Lethal	3	2(1)	4[a]	5[a]	2	1	8(2)	6	30		3	

( ) Indicate number of mutations from irradiated spermatogonia.

[a] One deletion involving both the *th* and *st* loci.

[b] An inversion is present in the third chromosome, but the breakage points do not involve the region of the mutated gene.

[c] Inversions are present with two of the mutations, but the breakage points do not involve the regions of the mutated genes.

[d] One mutation is associated with a shift; the shifted portion is also inverted.

tion" category, 14 of the 30 mutants were lethal or semilethal in the homozygous condition and 16 viable. All 13 deficiencies were lethal, as were all of the five translocations which were tested. The remaining three translocations involved the X or Y chromosome and were not tested for viability. All inversions survived in the homozygous condition; however, one was classed as a semilethal.

DISCUSSION

The X-irradiation damage to the genetic system as indicated by visible external morphological changes may or may not be associated with cytologically detectable damage to the chromosomes. From this and previous investigations it has been determined that visible mutations may be associated with no cytologically detectable changes or they may be associated with loss or rearrangement of chromosomal material, or with a combination of these chromosomal changes. We have used the term

"point mutation" throughout this paper for all mutations that are associated with no chromosomal changes detectable by the present cytological techniques, realizing that this does not rule out the possibility of submicroscopic loss or rearrangement of chromatin.

Genetic tests of the mutants permit a classification as to lethality, which may be correlated with the cytological analysis. Of the mutations analyzed in this investigation, a number of both the viable and lethal types showed no cytologically detectable changes. Almost equal numbers of visibles from mature sperm that were associated with no detectable structural changes were viable in the homozygous condition and lethal in the homozygous condition (16 viable, 14 lethal).

All thirteen cytologically detectable deficiencies were lethal in the homozygous condition. However, several cytological deficiencies that survived in the homozygous condition have been reported in the literature, among which are a four-band terminal deficiency in the X chromosome (DEMEREC and HOOVER 1936) and deficiencies for the yellow and achaete loci obtained by crossing over between the sc^8 and y^{3P} inversion (MULLER 1935).

The deficiencies analyzed in this investigation ranged in size from 3 to 38 bands on C. B. BRIDGES' map. They are: one 3-band, one 4-band, three 9-band, one 13-band, and seven deficiencies ranging from 20 to 38 bands. DEMEREC and FANO (1941) found that spontaneous Notch mutations were small deficiencies representing not more than 13 bands, but that X-ray-induced Notches had a much wider range. These data were interpreted to mean that those induced deficiencies of 13 bands or less were probably the result of single hits, whereas the larger deficiencies were caused by two hits. According to this interpretation, six of the 13 deficiencies that we studied would be due to single ionizing particles; however, MULLER (1954) is of the opinion that there are serious difficulties involved in such an interpretation (i.e., peculiarities of the Notch locus and origin of the spontaneous and induced deficiencies in different stages of the meiotic cycle). The work of VALENCIA and MULLER (1949) and VALENCIA (1952) indicates that deficiencies of more than three bands are induced by two hits. According to this interpretation, only one or possibly two of our deficiencies would be due to single hits.

Of the inversions associated with visibles, four were viable in the homozygous condition and one was semilethal. The five translocations tested in this investigation were lethal in the homozygous condition, but homozygous viable translocations have been reported (PATTERSON *et al.* 1934; BRIDGES and BREHME 1944). No cytologically detectable deficiencies were associated with the breakage points of any of the gross chromosomal changes of the type reported by SLIZYNSKI (1938).

The phenomenon of position effect must be considered in relation to those mutations associated with chromosomal aberrations of various types. LEWIS (1950), in his extensive review of the subject, classifies the position effect mutations into those which produce variegated phenotypes and those which produce stable phenotypes. None of the mutants studied in this investigation was observed to be variegated. The stable type usually involves the euchromatic region of the chromosome and the sensitive region is much narrower than for the variegated type. LEWIS points out that the X-ray-induced lethals associated with chromosomal aberrations have been

assumed to be position-effect mutants but cites evidence in support of the contention that only about 17% of these lethals would be due to position effects and that the remainder must be simultaneous breaks and point mutations. SUTTON (1943) has studied deficiencies that do not actually remove the loci of yellow and scute but influence the expression of those genes. Such position-effect deficiencies show the distance phenomenon in stable changes that must be taken into consideration in the placement of genes by all types of aberrations. In our investigation, 26 of the changes at visible loci were associated with chromosomal rearrangements or deficiencies. Crossover tests were carried out on these 26 mutants and one break of each aberration was found to be at or very near the locus in question. We are unable to determine which of these are position-effect mutations of the stable type and which are point mutations that are closely associated with chromosomal aberrations.

Table 4 gives a comparison of the data obtained by PAINTER and PATTERSON (1935) to the data from this investigation. As has been noted previously, PAINTER and PATTERSON used the *rucuca* stock, which carries eight recessive mutations in the third chromosome; the *res* stock used in this study carries the same mutants with the substitution of pink for claret. An X-ray dose of 3975r was used in the earlier experiment; 3000r was used in the present study. Since the other authors analyzed cytologically only those mutations that were lethal in the homozygous condition, only the lethal mutations from our results have been included in this table. A comparison of the percentages of each type of mutation shows fairly close agreement. In their investigation 40.9% of the lethal mutations were associated with no detectable chromosomal aberrations, and in the present investigation 42.4% were associated with no aberrations. Of the mutations studied cytologically, they found 31.8% that were associated with deficiencies and we found 39.4% to be associated with deficiencies. There is close agreement on the frequency of translocations with 13.6 and 15.2%, respectively. The largest variation is found in the frequency of inversions with 13.6% in their investigation and 3.0% in this study; however, these differences are not statistically significant.

In several other studies, genetic and cytological analyses of X-ray-induced mutations were carried out on recessive lethal mutations in a particular chromosome, on lethal mutations at particular loci, and in some instances on visible and lethal muta-

TABLE 4

A comparison of the data on the frequency of chromosomal aberrations associated with lethal mutations at eight specific loci in the third chromosome of D. melanogaster

Cytological analysis	Painter and Patterson (1935) 3975 r		Present Investigation 3000 r	
	Number	%	Number	%
No aberration	9	40.9	14	42.4
Deficiency	7	31.8	13	39.4
Inversion	3	13.6	1	3.0
Translocation	3	13.6	5	15.2
Total	22	99.9	33	100.0

tions at particular loci. OLIVER's work (1932) involved only a genetic analysis of sex-linked lethals, whereas SLIZYNSKI (1938) and SLIZYNSKA and SLIZYNSKI (1947), DEMEREC (1937), and SAKHAROV (1936) analyzed cytologically a number of X-ray-induced lethal mutations. DEMEREC (1937) analyzed lethal mutations that were associated with 17 different loci in the X chromosome. The mutations were collected over a number of years from experiments in which the dosage ranged from 2500 to 3000r. In 24 of the 61 cases (42.6%), an inversion or translocation occurred in the same chromosome with the lethal change. In 92.3% of these cases where a lethal change occurred in a chromosome with a gross chromosomal aberration, one of the break points coincided with the lethal mutation. In every case, the lethals affecting the specific loci were deficiencies, usually more than one band in size. In our investigation, we found equal numbers of lethal mutations that were or were not associated with deficiencies (13 with and 14 without detectable deficiencies). In the analysis of visible viable mutants DEMEREC found that only one out of 30 occurred in the chromosome with a gross chromosomal aberration, and in that instance neither of the break points coincided with the locus of the visible mutation. In contrast to these data, four out of 20 of the visible viable mutants that we analyzed were inversions in which one of the breaks was associated with the locus of the altered gene.

SLIZYNSKI (1938) reported that 30% of the X-ray-induced lethals were associated with deficiencies. This percentage of X-ray-induced mutations compares very well with the 31.8% obtained by PAINTER and PATTERSON and the 39.4% reported for our investigation. However, in the summary of the analyses of X-ray-induced sex-linked recessive lethal mutations reported by SLIZYNSKA and SLYZYNSKI in 1947, in which they considered the percentages of lethals that were associated with detectable deficiencies but not with gross structural rearrangements, there is a much greater difference in the proportions obtained. Of a total of 24 X-ray-induced lethal mutations from results obtained by SLIZYNSKI in 1938, 1942, and 1945 (unpublished data referred to by SLIZYNSKI), only 20.8% were associated with deficiencies as compared to 43.8% for PAINTER and PATTERSON's data and 48.2% for this investigation (excluding those lethal mutations associated with gross chromosomal aberrations). Although the difference between the data for the X chromosome loci and the lumped data for the specific autosomal loci is not statistically significant, attention is drawn to it because a larger sample might very well show a significant difference.

A comparison of X-ray-induced damage with ultraviolet- and fast neutron-induced damage has been dealt with by MULLER (1954) and by KAUFMANN (1954) and will not be discussed in this paper. However, we shall review briefly the data on spontaneous mutations that have been analyzed, since these data serve in the cytological comparisons for the present study. Any consideration of the cytological changes associated with spontaneous mutations is hampered by insufficient data, especially in the case of spontaneous mutations at specific loci. OLIVER (1932) tested 10 spontaneous lethal mutations by genetic methods and found none of them to be associated with chromosomal aberrations. SAKHAROV (1936) studied the salivary gland chromosomes of 25 spontaneous lethal mutations and found no chromosomal aberrations. Of the 45 spontaneous sex-linked lethal mutations analyzed genetically by DEMEREC (1937), none was associated with a rearrangement. SLIZYNSKI (1938) found that approximately half the spontaneous sex-linked lethal mutations that he studied

were associated with detectable deficiencies. In a cytological study of 19 spontaneous lethals, nine (47.3%) were deficiencies, whereas parallel study of 13 X-ray-induced lethal mutations revealed only four (30.0%). Thus there is no agreement on the frequency of the association of spontaneous lethal mutation with deficiencies, since SAKHAROV detected no loss of bands in the salivary gland chromosomes of those lethal mutations that he analyzed. From these analyses of 99 spontaneous lethal mutations, we see that none was associated with gross chromosomal aberrations. However, the spontaneous occurrence of gross chromosomal aberrations is known from the extensive studies on natural populations of various species of Drosophila in which large numbers of inversions have been detected in the natural populations. The spontaneous occurrence of reciprocal translocations is relatively rare, as evidenced by the very few instances in which this type of translocation has been detected in the large numbers of individuals from natural populations which have been analyzed cytologically (DOBZHANSKY and DREYFUS 1943; WARD 1952). The relative frequencies of translocations and inversions are not indicated by such data because of the possible differential selection between them in natural populations. The rare spontaneous occurrence of translocations is also evident from the *D. virilis* translocation tests (ALEXANDER and STONE 1955).

There are no previously reported cytological analyses of spermatogonial mutations. In our results, no two-break rearrangements were found associated with the spermatogonial mutations, whereas about one-half the mutations from mature sperm were associated with inversions, translocations, or deletions. Two-break rearrangements from premeiotic cells are rare, as shown by the radiation studies on males of *D. melanogaster* (AUERBACH 1954) and *D. virilis* (STONE *et al.* 1954; ALEXANDER and STONE 1955). Similar results were obtained in radiation studies on Drosophila females (GLASS 1955a, b) and on Sciara females (BOZEMAN and METZ 1949). More detailed cytological analyses of radiation damage in the prophase and metaphase of Habrobracon females have been reported by WHITING (1945a, b).

Considering only the genetic damage to the spermatogonia and mature sperm recovered as point mutations, there is some overlap of confidence intervals of the mutation rates. Since our data show that about one half of the mutations from mature sperm were associated with two-break rearrangements, an average rate of approximately 3×10^{-8}/r/locus can be estimated for point mutations in mature sperm; the 95% confidence interval of this rate would overlap the confidence interval of 0.728 to 2.79×10^{-8}/r/locus for the spermatogonial cells (ALEXANDER 1954). The importance of this overlap of confidence intervals depends on the interpretation of the induction of point mutations and chromosomal aberrations. If chromosomal aberrations are considered to differ from point mutations and to constitute an additional class, there is not a clear difference in the genetic damage recovered as point mutations from the two types of cells. On the mutation-by-breakage theory (where all mutations are dependent on chromosome breaks), this comparison of rates of induction of point mutations in the two stages would not necessarily imply a difference in mutation rates but may be due to differences in the fates of the chromosome breaks. The proportion of sex-linked lethals that were associated with chromosomal aberrations to those that were independent of aberrations (as measured by crossover inhibition) was shown by OLIVER (1932) to vary at different X-ray dosage levels. Al-

though LÜNING's data (1952) do not indicate an increase in the frequency of gynandromorphs, the visible mutations in postmeiotic cells do show a considerable increase. However, his data are not extensive enough to determine statistically whether or not the increase is comparable to that for dominant lethals. In LÜNING's tests, the visible mutations were not analyzed to determine the association with chromosomal breaks. The only proved findings concerning mutation and breakage in the various developmental stages of germ cells are the relatively frequent occurrence of both in the postmeiotic cells and the infrequent recovery of two-break rearrangements from premeiotic cells.

All spermatogonial mutations were classified cytologically as point mutations, but all three genetic classes, viable, lethal, and semilethal, were represented. Apparently there was no selection in spermatogonia or during gametogenesis against cells that were heterozygous for these new mutants. The absence of such selection is also evident in cases where the spermatogonial cells carry induced mutations that are almost completely lethal (e.g., *h* 200.3 ALEXANDER 1954). By a comparison to autosomal lethals, AUERBACH (1954) and earlier workers showed that there is selection against sex-linked lethals in the immature cells. The presence or absence of this type of selection is an important factor in the assessment of the relative genetic damage induced in spermatogonial and mature sperm cells. The present data do not indicate a selection against the spermatogonia carrying autosomal viable or lethal mutations when compared to the total spermatogonial population.

SUMMARY

Cytological studies have been made on 64 X-ray-induced mutations at eight specific loci in the third chromosome of *Drosophila melanogaster*. Among the mutations from mature sperm, 53.6% were associated with no chromosomal aberrations, 23.2% with deficiencies, 8.9% with inversions, and 14.3% with translocations. Of those 33 mutations which were lethal in the homozygous condition, 42.4% had no detectable chromosomal aberrations, 39.4% were deficiencies, 3.0% were inversions, and 15.2% were translocations.

All spermatogonial mutations were, cytologically, point mutations and represented all three viability categories. The recoverable genetic damage to the mature sperm is higher than that for spermatogonia when the calculations of the mutation rate for mature sperm is based on the total genetic damage (the sum of the cytologically determined point mutations and two-break aberrations). About one half of the mutations recovered from mature sperm were associated with chromosomal aberrations. When aberrations are omitted, the rates of point mutations for the two types of cells show an overlap of confidence intervals, but more adequate data may make the existing difference significant.

The aberrations were utilized to locate the eight loci cytologically on BRIDGES' standard salivary gland chromosome map.

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THE ANALYSIS OF ROD DERIVATIVES OF AN UNSTABLE RING CHROMOSOME OF DROSOPHILA MELANOGASTER[1]

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In contrast to those of corn, the ring chromosomes of *Drosophila melanogaster* are, with one exception, relatively stable. This exceptional Drosophila ring, called w^{vc}, is derived from a stable ring-X chromosome, and its instability is manifested through the frequent production of gynandromorphs, XO males, and dominant lethals among prospective w^{vc}/rod progeny. It has been proposed that these manifestations of w^{vc} instability are a consequence of anaphase bridge formation by the w^{vc} chromosome, whereby gynandromorphs and XO males arise from bridge loss, and dominant lethals owe their origin to bridge breakage (HINTON 1955). Since the work of MCCLINTOCK (1938) and SCHWARTZ (1953) suggests that sister-strand exchange is responsible for anaphase bridge formation by the ring chromosomes of corn, it appears that the same process might be the cause of w^{vc} instability. If this is true, the manifestations of instability should disappear upon conversion of the unstable w^{vc} ring chromosome to a rod chromosome because ring structure is a prerequisite for anaphase bridge formation as a consequence of sister-strand exchange. Studies on rod-w^{vc} chromosomes derived from unstable ring-w^{vc} chromosomes, as will be described in this paper, show that these rods are unstable. Although the manifestations of instability by w^{vc} rods are qualitatively different from those of the unstable ring-w^{vc} chromosome, they nevertheless are capable of interpretation in terms of anaphase bridge formation.

PRIMARY NONDISJUNCTION AND w^{vc} INSTABILITY

For interpretation of the results of crosses with w^{vc} rod chromosomes, one must first reconsider the relation of primary nondisjunction and instability of the w^{vc} ring chromosome. It was supposed (HINTON 1955) that patroclinous XO males produced by w^{vc}/rod females arose predominantly by w^{vc} loss during the meiotic divisions of the eggs and the early cleavage divisions of F_1 w^{vc}/rod zygotes. Primary nondisjunction was also recognized as a potential source of nullo-X eggs (and consequently XO males), but this source was assumed to be negligible since the frequency of recovered matroclinous females is very low in comparison with the patroclinous male frequency. However, if primary exceptional zygotes (w^{vc}/rod/Y) are subject to the dominant lethality experienced by their regular (w^{vc}/rod) sisters, the recovered matroclinous daughters represent only the surviving fraction of the w^{vc}/rod/Y zygotes produced. Consequently, a correction for dominant lethality applied to the observed matroclinous female frequency would provide a more reliable measure of primary nondisjunction frequency.

In each of the crosses listed in table 1 both primary exceptional females and

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TABLE 1

The relation between w^{vc} *instability and primary nondisjunction in crosses of* w^{vc}*/rod females. Details of calculations are found in the text*

Cross	Females					Males			Corrected percentage of exceptions		Index of w^{vc} instability (per cent)
	Regular			Matroclinous		Regular		Patrocli-nous			
	rod	w^{vc}	Gy	w^{vc}	Gy	rod	w^{vc}	rod (XO)	Female	Male	
Ia	1534	1263	11	0	0	1036	823	39	0.00	1.85	17.7
b	2832	920	393	2	2	2190	518	376	0.15	7.91	67.5
IIa	1498	642	158	21	6	1427	115	153	1.66	5.09	57.1
b	1055	261	142	7	5	1056	51	149	1.47	6.59	75.3
IIIa	6024	1961	524	81	42	4885	265	440	2.42	4.31	67.4
b	3796	487	279	25	30	2975	44	289	3.47	4.63	87.2
IVa	8919	7469	114	50	2	6859	1947	99	0.34	0.72	16.3
b	1490	582	297	8	7	1288	71	137	0.84	5.05	60.9
Va	4100	3141	33	2	0	3428	655	17	0.03	0.25	23.4
b	1383	438	315	22	24	1079	0	117	2.96	5.14	68.3

I. w^{vc}, *f/y w spl sn*^{3} × *y sc lz*^{9} *v f*/Y
II. w^{vc}, *f/y w spl sn*^{3} × dl-49, *y w v f car*/Y
III. w^{vc}, *f*/dl-49, *y w v f car* × *y w spl sn*^{3}/Y
IV. w^{vc}, *f*/dl-49, *y Hw m*^{2} *g*^{4} × dl-49, *y w lz*^{s}/Y
V. w^{vc}, *f*/dl-49, *y w B* × dl-49, *y w lz*^{s}/Y

gynandromorphs could be distinguished phenotypically from their regular sisters by appropriate combinations of mutant markers. In most of the crosses, the presence of the Y chromosome in primary exceptions could be detected by its suppression of eye color variegation exhibited by w^{vc}/w females. The corrected frequency of primary exceptional females (including gynandromorphs) is calculated as equal to (mat ♀ + mat Gy) ÷ 2(reg w^{vc} ♀ + reg Gy) + mat ♀ + mat Gy; it is assumed that the frequency of dominant lethals is equal among w^{vc}/rod and w^{vc}/rod/Y zygotes. The corrected frequency of primary exceptional males is calculated with the assumption that viability of XO males is equal to that of their regular rod/Y sibs so that the corrected frequency is equal to pat ♂ ÷ 2(reg rod ♂) + pat ♂; regular w^{vc}/Y males cannot be considered since their viability is probably affected by Notch variegation in addition to instability of the w^{vc} chromosome. Finally, an index of w^{vc} instability provided for each cross of table 1 is calculated as (reg rod ♀ − reg w^{vc} ♀) ÷ reg rod ♀. The sublines designated a and b for each of the crosses of table 1 were alike in all experimental conditions except for the w^{vc} chromosomes that were chosen from stocks having different degrees of instability. Comparisons within these sublines show that (except for Cross II) the corrected frequency of matroclinous females is higher in the subline having the higher instability index, which suggests that increased primary nondisjunction is still another manifestation of w^{vc} instability. In-

spection of the uncorrected data also provides evidence of this association between primary nondisjunction and instability.

Comparison of the corrected frequencies of primary exceptional females and males shows that for all crosses the male frequency is the higher. Since an excess of male over female exceptions is generally observed, the corrected frequency of exceptional females provides only a minimum estimate of exceptional males arising from primary nondisjunctional egg nuclei. However, different approaches are available to show that not all the exceptional males are the consequence of primary nondisjunction. Thus, on the anaphase bridge model of w^{vc} instability, bridge loss at the first cleavage mitosis produces only XO males whereas bridge loss at the second cleavage division is registered by gynandromorphs or, owing to the indeterminate distribution of early cleavage nuclei, by XO males. Another argument supporting the origin of XO males from w^{vc} loss is derived from the behavior of the w^{vc} chromosome when introduced by the male parent; in such crosses, XO males are also produced in frequencies similar to those found in progenies of w^{vc}/rod females, yet primary exceptional females (including gynandromorphs) are relatively rare in comparison. For example, the cross of *y w spl* sn^3 ♀ by w^{vc}, $f/sc^8 \cdot$Y ♂ produced 2469 *w spl* sn^3 ♂, 743 w^{vc} ♀, 151 Gy, 179 XO ♂ (table 1 of HINTON 1955); only one of the females and two of the gynandromorphs may have carried the $sc^8 \cdot$Y chromosome as judged by their phenotypes. From these considerations, it appears that both primary nondisjunction and w^{vc} loss are significant factors in the production of patroclinous XO males among the progeny of w^{vc}/rod females, although their relative contributions cannot be accurately evaluated.

THE CONSTRUCTION OF w^{vc} ROD CHROMOSOMES

The derivation of rods from ring-w^{vc} chromosomes is schematically outlined in figure 1. The structure of the w^{vc} ring chromosome was proposed (HINTON 1955) to be a long inversion with breakpoints between the loci of *y* and *w* and in the proximal heterochromatin. *In(1)*sc^4–sc^8 is a chromosome of inverted sequence combining the left end of *In(1)*sc^4 and the right end of *In(1)*sc^8 and lacking most of the basal heterochromatin. The B^s duplication is composed of the proximal part of the X chromosome through Bar and is derived from T(1; 4)B^s. At intervals for more than a year, females of the constitution w^{vc}, *f/y* sc^4 *car m* w^a sc^8/Dp B^s were synthesized and mass-mated to dl-49, *y w v f car*/Y males. The progenies were scored for all females (including gynandromorphs) carrying the w^{vc} chromosome. Among 11,259 w^{vc} females, 27 were found having the Bar eye and nonforked bristle phenotype expected for the rod-w^{vc} crossover product. On testing, six of these 27 females were sterile, 16 were of other double crossover or nondisjunctional origin, and five proved to be the desired crossovers of the constitution *y* sc^4 *car m* $w^{vc} \cdot y^+$ B^s/dl-49, *y w v f car*. These five rod-w^{vc} chromosomes, briefly designated $w^{vc} \cdot B^s$ (where the elevated dot indicates centromere position) and distinguished from each other by the catalog numbers of the cultures in which they occurred, were established in stocks for further testing.

Before describing the behavior of the $w^{vc} \cdot B^s$ chromosomes, let us consider the origin of derivatives of these chromosomes. In $w^{vc} \cdot B^s$/dl-49, *y w v f car* females, a single exchange between the B^s arm and the dl-49 chromosome will produce a com-

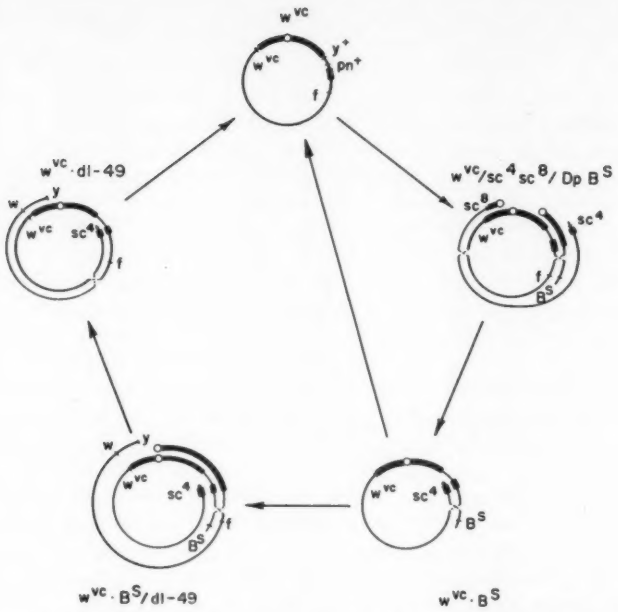

FIGURE 1.—The derivation of w^{vc} rod chromosomes and the generation of w^{vc} ring chromosomes from the rods. Details of the crosses involved are provided in the text. For simplicity, only two of the four chromatids of a tetrad are drawn, and the relative extents of heterochromatin (heavy line) and euchromatin (light line) are shown arbitrarily.

pound metacentric X chromosome of the constitution *y sc*^{4} *car m* w^{vc}·y^+ *f v w y*, dl-49 (abbreviated w^{vc}·dl-49) similar to the tandem metacentric heterozygous for the dl-49 inversion studied by NOVITSKI and BRAVER (1954). Several w^{vc}·dl-49 chromosomes were obtained from each of the five w^{vc}·B^s stocks. Neuroblast preparations confirmed the metacentric structure of both the w^{vc}·B^s and w^{vc}·dl-49 chromosomes. It is of importance to note that both these rod-w^{vc} chromosomes retain intact the centromere and associated heterochromatic regions of the w^{vc} ring chromosome because some component of these regions appears to be responsible for w^{vc} instability (HINTON 1955).

Inspection of the pairing configurations of the w^{vc} rod chromosomes shows that a single exchange between the two arms of w^{vc}·B^s or w^{vc}·dl-49 chromosomes leads to the generation of w^{vc} ring chromosomes (only half the indicated singles would generate rings since, at the four-strand stage, chromatids having a common centromere must be involved in the exchange). These generated w^{vc} rings were recovered from both the w^{vc}·B^s and w^{vc}·dl-49 chromosomes from each of the five stocks. Since the original w^{vc}/sc^4-sc^8/Dp B^s females were produced and mated in mass cultures, nothing is known about the initial instability of the individual rings that gave rise to the w^{vc}

rods; but the behavior of the generated w^{vc} rings, identical in structure to the originals, provides essential control information on this point.

THE BEHAVIOR OF w^{vc} ROD CHROMOSOMES

The results of crosses involving the $w^{vc} \cdot Bs$, $w^{vc} \cdot$dl-49 and generated w^{cc} ring chromosomes are presented in tables 2, 3 and 4. The presentation of artificial (reconstructed) tetrad analyses for these chromosomes is unwarranted since the structural and behavioral complexities involved require rather questionable assumptions and cumbersome manipulations. Instead, quantitative considerations of the results rely on comparisons of arbitrarily based frequencies of the various classes of offspring recovered.

The very rare gynandromorphs listed in table 2 from crosses of $w^{vc} \cdot Bs$/dl-49 females show that none of the five $w^{vc} \cdot Bs$ chromosomes experience somatic loss frequencies that are at all comparable to those exhibited by unstable rings. By this criterion alone, we would conclude that the $w^{vc} \cdot Bs$ chromosomes are stable. However, if the $w^{vc} \cdot Bs$ female and XO male frequencies (based on the number of $y\ w$ females) are examined, the five $w^{vc} \cdot Bs$ stocks can be separated into two groups. Although minor differences between stocks may be found within either group, the results from each stock have been combined to form the group total in order to facilitate comparisons. In the group consisting of stocks designated 5374, 5582, and 6850 there is a deficiency of $w^{vc} \cdot Bs$ females compared to the approximate 1:1 expectation for $y\ w$

TABLE 2

The behavior of $w^{vc} \cdot B^s$ *chromosomes in crosses of* $w^{vc} \cdot B^s$*/dl-49,* y w lz^s *females to dl-49,* y w v f car/*Y males*

$w^{vc} \cdot B^s$ stock number	$y\ w\ lz^s$ males	$y\ w$ females	Regular $w^{vc} \cdot B^s$ females	Matroclinous $w^{vc} \cdot B^s$ females*	Gy	$y\ w\ f$ males	Generated rings			Generated compounds w^{vc}·dl-49
							w^{vc} females	Gy	w^{vc} males	
5374	919	1222	1041	—	0	4	0	1	2	0
5582	2087	2880	2423	—	1	13	7	0	8	2
6850	1015	1364	1159	—	0	9	5	0	4	4
Total	4021	5466	4623	—	1	26	12	1	14	6
Percent		100.0	84.6	—	0.0	0.5	0.2			0.1
6848	1730	2545	1219	—	3	120	2	5	0	4
6857	1477	2095	1054	—	5	94	3	0	1	5
Total	3207	4640	2273	—	8	214	5	5	1	9
Percent		100.0	49.0	—	0.2	4.6	0.2			0.2
6850	1228	2117	1659	8	0	6	8	0	5	6
Percent		100.0	78.4	0.4	0.0	0.3	0.4			0.3
6857	838	1668	856	69	0	134	1	2	0	1
Percent		100.0	51.3	4.1	0.0	8.0	0.2			0.1

* Distinguished from regular females by the effect of the Y chromosome on eye color variegation.

and $w^{vc} \cdot B^s$ females. Part of this deficiency arises through w^{vc} ring and compound generation, but the greater part is probably attributable to hyperploidy of the B^s segment of the chromosome (which also partially accounts for the semilethality of $w^{vc} \cdot B^s$ males). In the second group, consisting of the two stocks numbered 6848 and 6857, the deficiency of $w^{vc} \cdot B^s$ females is about three times as great as in the first group, although inviability owing to hyperploidy should be the same in the two. The net difference between the $w^{vc} \cdot B^s$ female frequencies for the two groups of 35.6% is quantitatively comparable to the frequency of dominant lethals caused by the unstable ring chromosomes.

Similarly, the tenfold difference in XO male frequency between the two groups of $w^{vc} \cdot B^s$ stocks parallels that difference between stable and unstable w^{vc} ring stocks. Since neither of the $w^{vc} \cdot B^s$ stocks numbered 6848 and 6857 produce significant numbers of gynandromorphs, it is not reasonable to suppose that the XO males produced by these same stocks are the consequence of somatic loss of the $w^{vc} \cdot B^s$ chromosome. An alternative—that these XO males are chiefly of primary nondisjunctional origin—is supported by the observations on the frequency of primary exceptional daughters produced by $w^{vc} \cdot B^s$ females (matroclinous $w^{vc} \cdot B^s$ females could be accurately distinguished from their regular sisters as shown by progeny tests). Comparison of the data from stocks 6850 and 6857 (lower part of table 2) shows that the frequencies of patroclinous males and matroclinous females are positively correlated.

In the $w^{vc} \cdot$dl-49 crosses (table 3), the $sc^8 \cdot$Y chromosome was utilized to distinguish regular (XY) males from their exceptional (XO) sibs, and the dominant *B* served as a convenient marker for the detection of females bearing generated ring chromosomes. Although superfemale-XO male mosaics might be expected as a consequence of $w^{vc} \cdot$dl-49 somatic loss, no mosaics of this type were positively identified, indicating that none of the $w^{vc} \cdot$dl-49 compounds experience somatic loss. However, examination of the frequencies of the various offspring classes, based on the incidence of *y w B/*

TABLE 3

The behavior of $w^{vc} \cdot$*dl-49 compound chromosomes in crosses of* $w^{vc} \cdot$*dl-49/*$sc^8 \cdot$Y *females to dl-49,* y w B/*Y males*

$w^{vc} \cdot B^S$ stock number	Number of compounds tested	*w B* males	w^{vc} females	*y w B* males	Generated rings		
					w^{vc} *B* females	Gy	w^{vc} males
5374	3	534	437	4	50	0	29
5582	2	869	807	7	61	1	52
6850	3	618	551	7	53	0	11
Total		2021	1795	18	164	1	92
Percent		100.0	88.8	0.9	8.2		
6848	3	1045	632	31	42	16	13
6857	7	2808	1439	93	119	47	13
Total		3853	2071	124	161	63	26
Percent		100.0	53.8	3.2	5.8		

$sc^8 \cdot Y$ males, shows that the $w^{ec} \cdot$dl-49 chromosomes are also separable into two groups corresponding with the two categories of $w^{ec} \cdot B^s$ chromosomes. Comparison of the first group (derived from $w^{ec} \cdot B^s$ stocks 5374, 5582, and 6850) with the second group (derived from $w^{ec} \cdot B^s$ stocks 6848 and 6857) reveals significant differences in the recovery of females bearing $w^{ec} \cdot$dl-49 chromosomes and in the incidence of XO males. The 35% deficit of $w^{ec} \cdot$dl-49 females in the second group suggests that zygotes bearing these chromosomes are also susceptible to the dominant lethality experienced by unstable ring w^{ec} chromosomes.

The assignment of the origin of the XO males produced by the $w^{ec} \cdot$dl-49/$sc^8 \cdot Y$ females requires consideration of several potential sources. (1) Since no superfemale-XO male mosaics were observed, somatic loss of the $w^{ec} \cdot$dl-49 chromosome apparently cannot account for XO male production. (2) Of the interarm exchanges occurring in either the $w^{ec} \cdot B^s$ or $w^{ec} \cdot$dl-49 chromosomes, only those that involve chromatids having a common centromere will generate rings; the other half of such exchanges will cause the production of a single chromatid bridge at the second meiotic anaphase. If this bridge were lost and the functional egg nucleus fertilized by an X-bearing sperm, an XO male would be produced. However, this is not the case, as shown by the high frequency of generated rings and low frequency of XO males produced by females carrying $w^{ec} \cdot$dl-49 compounds derived from $w^{ec} \cdot B^s$ stocks 5374, 5582, and 6850. This result agrees with that of NOVITSKI and BRAVER (1954) from their study of the compound metacentric heterozygous for dl-49 and supports their conclusion that such bridges suffer breakage rather than loss. (3) If some mechanism other than interarm exchange (see DISCUSSION) should lead to second meiotic anaphase bridge formation by the $w^{ec} \cdot$dl-49 chromosome, it seems probable that these bridges also would be broken rather than lost. (4) Another possible source of XO males involves nondisjunction of the $w^{ec} \cdot$dl-49 and $sc^8 \cdot Y$ chromosomes to produce nullo-X-Y and $w^{ec} \cdot$dl-49/$sc^8 \cdot Y$ egg nuclei. Fertilization of the first type of egg nucleus by an X-bearing sperm would produce an XO male. If the latter type egg nucleus were fertilized by a Y-bearing sperm, $w^{ec} \cdot$dl-49/$sc^8 \cdot Y/Y$ females should be produced. From the cross of $w^{ec} \cdot$dl-49/$sc^8 \cdot Y$ females (derived from $w^{ec} \cdot B^s$ stock 6857) to dl-49, *y w* B/Y males, 259 F_1 females were progeny tested and none carried the $sc^8 \cdot Y$ chromosome in addition to the unmarked Y chromosome. This result does not conclusively deny the occurrence of nondisjunction since it is possible that $w^{ec} \cdot$dl-49/$sc^8 \cdot Y/Y$ zygotes will not survive; on the other hand, this result is comparable to the apparently negligible nondisjunction frequency in unstable $w^{ec}/sc^8 \cdot Y$ males. (5) The most probable origin of XO males is somatic loss of generated ring chromosomes. In the group of $w^{ec} \cdot$dl-49 chromosomes characterized by a low (0.9%) XO male frequency, only one of 165 generated ring females was a gynandromorph; in the other group having an XO male frequency of 3.2%, 63 of 224 generated ring females were gynandromorphs. When generated rings undergo somatic loss to produce gynandromorphs, they may be expected to produce XO males by the same process.

From the data just considered on gynandromorph frequencies among generated ring females, it is clear that the two groups of $w^{ec} \cdot$dl-49 chromosomes are distinguishable on this basis as well as by $w^{ec} \cdot$dl-49 female recovery frequencies. This conclusion may be strengthened and extended to the $w^{ec} \cdot B^s$ chromosomes by study

TABLE 4

The behavior of generated w^{vc} *ring chromosomes (from* $w^{vc}\cdot B^S$ *and* w^{vc}*·dl-49 rods) in crosses of* w^{vc}*/dl-49,* y w lz^a *females to dl-49,* y w v f car/*Y males*

$w^{vc}\cdot B^S$ stock number	Number of rings tested	w^{vc} males	y w lz^a males	y w females	w^{vc} females	Gy	y w *f* males
5374	8	138	403	442	444	16	8
5582	18	640	954	1336	1174	20	10
6850	6	7	291	357	395	3	0
Total		785	1648	2135	2013	39	18
Percent				100.0	94.3	1.8	0.8
6848	11	183	628	819	572	88	36
6857	23	60	865	1624	492	250	130
Total		243	1493	2443	1064	338	166
Percent				100.0	43.5	13.8	6.8

of the generated rings in subsequent generations. Ring-w^{vc} chromosomes generated directly by the $w^{vc}\cdot B^S$ chromosomes or indirectly via the w^{vc}·dl-49 chromosomes were collected and established in stocks for further testing; the results of these tests (table 4) are pooled according to the five original $w^{vc}\cdot B^S$ stocks. There are two distinct groups of generated rings: those derived from $w^{vc}\cdot B^S$ stocks 5374, 5582, and 6850 are nearly stable, as shown by low frequencies of gynandromorphs and XO males and by high frequencies of w^{vc} female recovery; those derived from $w^{vc}\cdot B^S$ stocks 6848 and 6857 are unstable, as shown by high gynandromorph and XO male frequencies and by low recovery of w^{vc} females. On the basis of these and other observations, the behavior of these two groups of generated rings is entirely comparable to that of the stabilized and unstable w^{vc} ring chromosomes previously described (HINTON 1955).

In summary, none of the derived rod-w^{vc} chromosomes are unstable, by the criterion of somatic elimination, since no sex mosaics are recovered and since the XO males that do appear are attributable to other sources. However, other manifestations of w^{vc} instability allow the separation of the derived w^{vc} rods into two groups. Both the $w^{vc}\cdot B^S$ and w^{vc}·dl-49 chromosomes that generate unstable w^{vc} rings are characterized by significantly reduced recovery frequencies (dominant lethality) compared to those rod-w^{vc} chromosomes which generate stable rings. In addition, the $w^{vc}\cdot B^S$ chromosomes that generate unstable rings exhibit significantly higher primary exceptional male and female frequencies than those that generate stable rings, again paralleling the behavior of stable and unstable rings. It is concluded on the basis of the dominant lethality and primary nondisjunction manifestations of instability that rod-w^{vc} chromosomes may be either stable or unstable depending, inferentially, on whether they were derived from stable or unstable ring-w^{vc} chromosomes.

DISCUSSION

Interpretation of the experiments described in this report assumes the validity of the anaphase bridge model of w^{vc} instability. This model, as formulated to explain the instability manifestations of the ring-w^{vc} chromosomes, proposes that an ana-

phase bridge may suffer either of two fates. It may be lost; whereupon a prospective female zygote will be converted to an XO male or to a gynandromorph. Alternatively, the bridge may break, whereupon delivery of the breakage products to the daughter nuclei will usually be lethal to the zygote. These experiments also rely on the thesis that sister-strand crossing over will lead to anaphase bridge formation in a ring chromsome but will be of no consequence in a rod chromosome. If anaphase bridges formed by a ring chromosome continue to be formed when the ring is converted to a rod, then the cause of bridge formation cannot be sister-strand exchange.

For the anaphase bridge mechanism to be operative in rod-w^{ec} chromosome instability, some modification of the model is necessarily since the rod-w^{ec} chromosomes show no evidence of somatic loss; this result requires that the anaphase bridges formed by rods always break, producing dominant lethals. Application of the anaphase bridge model to rod-w^{ec} instability further requires that sister-strand exchanges be discarded as the process responsible for bridge formation. An alternative possibility is union between sister chromatids, a process comparable with that producing the reversed acrocentric compound X chromosome (SANDLER 1954); in its frequency and variability, sister-strand union in the w^{ec} chromosomes may be more appropriately compared to certain of the *Ds* events described by MCCLINTOCK (1951) in corn. If sister-strand union occurs in a chromosome of ring structure, the resultant bridge would be composed of two chromatids, just as in sister-strand exchange, except that the distribution of genetic material in the bridge would be different. Whether or not bridges arising from sister-strand union are symmetrical, that is to say, composed of ties of equal strength, would depend on the position of the union. Union between sister strands of a rod chromosome would produce a bridge having centromeres connected by only a single chromatid, plus an acentric fragment. Either loss of the acentric fragment or breakage of the bridge, or a combination of both events, is presumably sufficient cause for zygotic lethality. Following NOVITSKI's analysis (1955) of meiotic anaphase bridge behavior, the difference in somatic behavior of rod-w^{ec} and ring-w^{ec} chromosomes can be visualized as follows: sister centromeres, connected by two chromatids of the same strength, may not always be able to break both chromatids, so that the entire bridge will sometimes be lost, but when these centromeres are connected by only a single chromatid breakage always occurs.

Brain smears of larval $w^{ec} \cdot B^s$ and $w^{ec} \cdot$ dl-49 females were prepared to confirm the metacentric structure of these chromosomes, but in the few smears examined, no definite anaphase bridges were found. One might suppose that anaphase bridges are formed by unstable w^{ec} rods in larval brain tissue but that bridges composed of single chromatids are either so short or so weak that they fracture during early anaphase movements and so escape notice. Nevertheless, the predicted acentric fragment, which should be visible at later anaphase and telophase stages, was not seen in the few configurations suggestive of bridges or in other anaphases. This negative preliminary result is of questionable significance for even if large numbers of smears were examined, the absence of bridges in brain mitoses would not rule out their occurrence in cleavage or meiotic divisions.

The observation of bridges at the second meiotic anaphase of w^{ec} males (WELSHONS and HINTON 1955) supported the previously suggested possibility that XO males

and dominant lethals originate from meiotic as well as cleavage bridges formed by the unstable ring chromosomes. Genetic evidence in support of the meiotic origin of dominant lethals is provided by the recovery frequencies of rings generated by the w^{ec}·dl-49 compounds (table 3). Considerations presented earlier indicated the most probable source of XO males produced by w^{ec}·dl-49 females to be somatic loss of generated rings. Therefore, these XO males should be added to the generated ring female and gynandromorph classes to provide a measure of the total ring generation frequency, which amount to 9.1% for the stable and 9.0% for the unstable compounds. Apparently, none of the zygotes receiving newly generated rings experience dominant lethality. This result suggests that dominant lethals caused by unstable ring chromosomes are entirely of meiotic origin. If this interpretation is correct, it follows that the occurrence of an interarm exchange of the type that generates a ring precludes the subsequent occurrence of a sister-strand union necessary to produce a second anaphase bridge. Another corollary of the interpretation that dominant lethals arise solely from meiotic bridges is that somatic bridges formed by rings do not fracture. However, somatic bridge breakage is demanded by the occurrence of fragments of the w^{ec} ring in gynandromorphs (HINTON 1955). Because of the limited and apparently contradictory evidence, further discussion of this question is not justified.

It seems unlikely that anaphase bridge formation could account for the increase of primary exceptional females associated with increased instability as defined by chromosome loss and dominant lethality. According to SANDLER and BRAVER (1954), primary nondisjunction is best explained by failure of pairing in such a way that homologs proceed to the first anaphase poles at random. COOPER (1951) presented cytological evidence and NOVITSKI and BRAVER (1954) obtained genetic data supporting the view that homolog pairing is initiated by special sites located in the proximal heterochromatin of the X chromosome. It may be inferred that abnormality of this region would lead to inefficient pairing and nondisjunction. Nothing is known of the detailed heterochromatic structure of the w^{ec} chromosome; it is probable that the w^{ec} centromere is of Y chromosome origin, that at least some of the heterochromatic components are duplicated and that these are rearranged in relation to the structure of X^{ca}. Some element of this region of the w^{ec} chromosome controls its instability. This conclusion is based on the continued high somatic loss frequency of fragments derived from and comprising chiefly some of the heterochromatin of the unstable w^{ec} ring (HINTON 1955). It is suggested that the element of the w^{ec} chromosome controlling anaphase bridge formation is also responsible for meiotic pairing failure.

SUMMARY

Data have been presented that show increased primary nondisjunction to be another attribute of the unstable w^{ec} chromosome. It is suggested that primary nondisjunction is a consequence of some defect of the heterochromatic region of the w^{ec} chromosome that prevents normal homolog pairing. Previous evidence indicated that some heterochromatic element of this chromosome controls formation of anaphase bridges, which provide the basis for other manifestations of w^{ec} instability.

The primary purpose of this work, to test whether sister-strand exchange causes anaphase bridge formation by the unstable w^{vc} ring chromosome, was implemented by analysis of rod chromosomes derived from w^{vc} ring chromosomes. Although the rod-w^{vc} chromosomes do not experience somatic loss, they may be unstable by the criterion of dominant lethality. This result can be explained by assuming that sister-strand union rather than sister-strand exchange leads to anaphase bridge formation, and that a bridge formed by a ring chromosome and consisting of two chromatids is susceptible to either loss or breakage; whereas a bridge formed by a rod chromosome and composed of a single chromatid is always broken.

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GENE CONTROL OF RESISTANCE VS. SENSITIVITY TO ACTIDIONE IN SACCHAROMYCES[1]

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Actidione was shown by WHIFFEN (1948) to have marked antibiotic activity against yeasts but little or no activity against bacteria. The observation that sensitivity vs. resistance to copper (BRENES-POMALES *et al.*) and cadmium (MIDDLEKAUFF *et al*, is under gene-control in Saccharomyces led to an attempt to discover if sensitivity vs. resistance to antibiotics is also gene-controlled.

MATERIALS AND METHODS

A modified Burkholder's medium (table 1) autoclaved at 15 lbs. pressure for 15 minutes after being adjusted to pH 5 was used in 10 ml amounts in broth tubes, or 3% agar was added for use in petri dishes. Actidione stock solution (10 mg of actidione in 100 ml of water) was sterilized by Seitz filtration, kept in a refrigerator until used, and added aseptically to the medium after autoclaving.

One loopful of cells from a 24 hour culture was transferred to a 6 ml water blank to make a final turbidity of about 150 Klett units. One loopful of this suspension containing about 60,000 cells was used to inoculate a broth tube or planted on a spot about 1 cm in diameter on an agar plate. Tubes and plates were incubated at 30°C. Results of growth in tubes were recorded after each 24-hour incubation (up to 96 hours) with a Klett Summerson Photoelectric colorimeter, using a 420 mμ blue filter, with the zero point adjusted against an uninoculated broth tube. Growth on agar was recorded at four different quantitative levels; 0, no growth; 1, discrete colonies; 2, irregularly confluent growth; and 3, fully confluent growth.

EXPERIMENTAL RESULTS

Ten cultures of Saccharomyces from the Carbondale breeding stock were tested on agar plates containing various concentrations of actidione from 0.7 to 1.0 parts per million. Two cultures showed weak but confluent growth on 0.7 ppm and a few small colonies on 1.0 ppm; the others failed to grow. A resistant colony of culture 18159 (18159R) was picked from a 1.0 ppm plate. It grew vigorously at 1.2 ppm. Family I (fig. 1) was established by crossing 18159R to a sensitive strain.

Tests on solid and liquid medium (table 2) revealed that yeast cells were inhibited by lower concentrations of actidione in liquid than in solid medium, especially when the inoculum on solid medium was large and many cells were planted in a restricted area. This was interpreted to indicate that the local concentration of actidione was probably reduced by the absorption of the toxic agent by cells which had died, when

[1] This investigation was supported by a research grant from the Illinois Division of the American Cancer Society.

TABLE 1

Modified Burkholder's medium (per liter)

SALTS	
$(NH_4)_2SO_4$	4 g
KH_2PO_4	1.5 g
$MgSO_4$	0.5 g
$CaCl_2$	0.33 g
$FeSO_4 \cdot 7H_2O$	2.5 mg
$ZnSO_4 \cdot 7H_2O$	3.1 mg
$CuSO_4 \cdot 5H_2O$	0.4 mg
KI	10 mg
$MnSO_4 \cdot 4H_2O$	0.4 mg
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.18 mg
$Na_2B_4O_7 \cdot 10H_2O$	0.88 mg
SUGAR SOURCE	
glucose	20 g
AMINO ACIDS	
Proline, arginine, leucine, lysine, isoleucine, histidine, methionine, phenylalanine and anthranilic acid, each in 0.0002 M final concentration.	
Asparagine	2 g
N-BASES	
Adenine sulfate	40 mg
Uracil	20 mg
VITAMINS	
Thiamine-HCl	200 mcg
Nicotinic acid	200 mcg
Paba	50 mcg
Pyridoxine	200 mcg
Ca pantothenate	200 mcg
Inositol	1000 mcg
Biotin	2 mcg

many cells had been planted on solid medium. The diffusion of more toxin into the region was probably slow and the surviving cells were exposed to concentrations of actidione lower than the original. A series of tests were designed to discover the tolerances of actidione by resistant and sensitive cultures and to determine the influence of cell numbers upon the inhibitory effect of actidione. The four cultures of an ascus in which regular segregation had occurred for sensitivity and resistance were inoculated into broth tubes and agar plates at concentrations from 0 to 3.0 ppm. Loops of cell suspensions containing numbers ranging from 3.7×10^3 to 2.5×10^5 cells were used as inocula.

The plus (+) and minus (−) signs in table 2 (instead of density readings) record the results of the tests in *liquid* medium. The lowest density readings in the (+) categories were about 150 as compared to over 900 for the tubes in which abundant growth occurred, but the (−) categories uniformly showed no growth. Although culture 18718 is clearly a sensitive culture, it grew at 0.2 ppm when the inoculum was heavy. Culture 18720 (which is also sensitive) failed to grow in every instance in liquid medium at 0.2 ppm. At cell concentrations ranging from 2.5×10^5 to $3.0 \times$

TABLE 2

Diagnosis in liquid and in solid medium

Cultures	Numbers of Cells	Actidione Concentration in PPM.							
		0	0.2	0.5	0.8	1.0	1.5	2.0	3.0
18717	2.5×10^5	+/3	+/3	+/3	+/3	+/3	+/3	+/3	−/2
18718	2.5×10^5	+/3	+/2	−/1	−/0	−/0	−/0	−/0	−/0
18719	2.5×10^5	+/3	+/3	+/3	+/3	+/3	+/3	+/3	−/2
18720	2.5×10^5	+/3	−/3	−/1	−/0	−/0	−/0	−/0	−/0
18717	1.2×10^5	+/3	+/3	+/3	+/3	+/3	+/3	−/3	−/2
18718	1.2×10^5	+/3	+/2	−/1	−/0	−/0	−/0	−/0	−/0
18719	1.2×10^5	+/3	+/3	+/3	+/3	+/3	−/3	−/3	−/2
18720	1.2×10^5	+/3	−/3	−/1	−/0	−/0	−/0	−/0	−/0
18717	6.0×10^4	+/3	+/3	+/3	+/3	+/3	−/3	−/2	−/1
18718	6.0×10^4	+/3	+/2	−/1	−/0	−/0	−/0	−/0	−/0
18719	6.0×10^4	+/3	+/3	+/3	+/3	+/3	−/3	−/3	−/1
18720	6.0×10^4	+/3	−/3	−/0	−/0	−/0	−/0	−/0	−/0
18717	3.0×10^4	+/3	+/3	+/3	+/3	+/3	−/3	−/2	−/1
18718	3.0×10^4	+/3	+/1	−/0	−/0	−/0	−/0	−/0	−/0
18719	3.0×10^4	+/3	+/3	+/3	+/3	+/3	−/3	−/2	−/0
18720	3.0×10^4	+/3	−/3	−/0	−/0	−/0	−/0	−/0	−/0
18717	1.5×10^4	+/3	+/3	+/3	+/3	+/3	−/1	−/2	−/1
18718	1.5×10^4	+/3	−/1	−/0	−/0	−/0	−/0	−/0	−/0
18719	1.5×10^4	+/3	+/3	+/3	−/3	−/3	−/1	−/2	−/0
18720	1.5×10^4	+/3	−/2	−/0	−/0	−/0	−/0	−/0	−/0
18717	7.5×10^3	+/3	+/3	+/3	−/3	−/3	−/1	−/1	−/0
18718	7.5×10^3	+/3	−/1	−/0	−/0	−/0	−/0	−/0	−/0
18719	7.5×10^3	+/3	+/3	+/2	+/3	−/3	−/1	−/2	−/0
18720	7.5×10^3	+/3	−/1	−/0	−/0	−/0	−/0	−/0	−/0
18717	3.7×10^3	+/3	+/3	+/3	−/3	−/3	−/1	−/1	−/0
18718	3.7×10^3	+/3	−/1	−/0	−/0	−/0	−/0	−/0	−/0
18719	3.7×10^3	+/3	+/3	+/2	−/3	−/3	−/1	−/2	−/0
18720	3.7×10^3	+/3	−/1	−/0	−/0	−/0	−/0	−/0	−/0

10^4 and at concentrations of actidione ranging from 0.5 ppm to 1.0 ppm, the gene-pair principally responsible for differentiating sensitivity and resistance are clearly distinguished. The regularity of segregation indicates (fig. 1) that the principal genes are stable.

The numbers (table 2) show the results of tests on *solid* medium. Diagnosis is possible in every instance at 2 ppm even with the smallest number of cells. Growth occurred on every spot at concentrations of 0.2 ppm. There is general agreement between tests in liquid and solid media, but cells grow on solid medium at concentrations of actidione which are inhibitory in liquid medium. Discrete colonies are

TABLE 3

Replicate tube tests

Cultures	Days	Turbidity Readings						
		1	2	3	4	5	6	7
18717	2	10	45	15	45	15	15	30
	3	220	130	300	105	240	300	375
18718	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
18719	2	35	50	75	70	55	115	95
	3	240	240	260	260	230	300	290
18720	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0

formed in the area of the drop when sensitive cultures are planted on low concentrations of actidione, indicating that a few cells from a sensitive culture can grow on low concentrations of actidione. Discrete colonies also appear when resistant cells are planted on high concentrations of actidione. This suggests that the populations of both resistant and sensitive inocula are usually mixed. As long as the cultures are tested, without previous contact to actidione, in liquid medium containing 1 ppm with an inoculum of about 6×10^4 cells per tube, a differentiation between the genes controlling sensitivity and resistance can be demonstrated.

Table 3 records the results of a series of replicate tube tests read after two and three days in which 1 ppm of actidione was used with an inoculum of 6×10^4 cells per tube. The two sensitive cultures failed to grow; all resistant cultures grew in every tube with minor variations in amounts of growth. This finding supports the use of the tube test for distinguishing the two members of this gene-pair.

Six heterozygous families (I to VI, fig. 1) were analyzed from crosses made between actidione-resistant and actidione-sensitive cultures. The expected segregations occurred in 68 of the 71 tetrads from the heterozygotes. The actidione-resistant parental cultures for Families II, IV, V, and VI were descended from the resistant mutant 18159R which was the actidione-resistant parent in Families I and III. The actidione-sensitive parental cultures for the first five families were all obtained from the breeding stock (outside the pedigree). The actidione-sensitive parental culture of Family VI was selected from Family IV.

A homozygous mating (Family VII of two actidione-sensitive strains, chosen from Family II) produced segregants all of which were sensitive to actidione.

Family VIII arose from a cross made between an actidione-sensitive strain from Family II and an actidione-sensitive strain selected from outside the pedigree. It produced offspring which were all sensitive to actidione.

A homozygous mating (Family IX) of two actidione-resistant cultures, both of which were obtained from Family II, yielded tetrads all resistant to actidione.

Every family was heterozygous for from two to six other genes which generally segregated regularly. There was no evidence of linkage between AC and other genes.

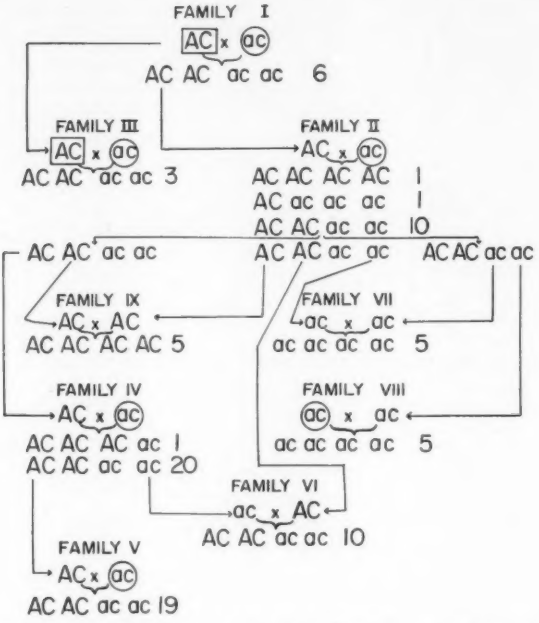

FIGURE 1.—Pedigree of Actidione Sensitivity and Resistance. The symbols AC and ac signify resistance and sensitivity respectively. The inscribed symbols indicate cultures selected from the breeding stock. The numerals represent the number of different types of tetrads resulting from each mating.

Three diploid zygotes of the same genotype as the hybrid from which Family I (fig. 1) originated:

17911	*α*	*su*	*GA ME*	*ma*	*CD MG*	*ac*
18159R	*a*	*su*	*GA me*	*MA*	*cd mg*	*AC*

were tested for fermentative ability and found to be suc GAL MAL MGS MEB. The two-letter symbols indicate genotypes (small letters for recessive and capitals for dominants): *SU*, sucrose-fermentation; *GA*, galactose-fermentation; *MG*, methyl alpha-D-glucopyranoside-fermentation; *ME*, melibiose-fermentation; *CD*, cadmium-resistance. Three-letter symbols indicate phenotypes. Capitals indicate fermentative ability (or resistance) and small letters inability to ferment (or sensitivity).

This confirms the view that the isolates were diploid. The diploid cultures were tested under the conditions which discriminate resistance and sensitivity of haploid cultures to cadmium and actidione and found to be "resistant." It was inferred that the genes controlling resistance to cadmium and actidione are dominant although all the implications of this inference have not yet been explored completely.

A series of transfers on agar containing high concentrations of actidione yielded a few discrete colonies which grew on agar containing 50 ppm of actidione. The discrete colonies were all restricted to a single drop and presumably originated from a single clone.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the gift of actidione from the Upjohn Company.

SUMMARY

A single gene-pair in Saccharomyces controlling sensitivity versus resistance to actidione has been described. The optimal conditions for diagnosis of the genotype is liquid culture at 1 ppm of actidione inoculated with 6×10^4 cells. The relations between the size of the inoculum and the concentration of actidione (as well as the effect of testing on solid and liquid medium) were observed.

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MORPHOGENETIC STUDIES OF THE RABBIT. XVII. DISPROPORTIONATE ADULT SIZE INDUCED BY THE DA GENE.[1]

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Extensive studies of chondrodystrophia foetalis in the rabbit have been described by BROWN and PEARCE (1945) and PEARCE and BROWN (1945a and 1945b), which show it to be due to a simple Mendelian recessive gene which induces the characteristics typical of human chondrodystrophy. Homozygous individuals die at or within a few hours of birth.

In a previous communication we have dealt with the gross external manifestations and simple hereditary basis of a second type of so-called "achondroplasia" (dachs) in the rabbit (CRARY and SAWIN, 1952). Although originally reported as a recessive, certain of its pleiotropic characteristics to be described here are manifest in heterozygotes, and in some cases exceed either parental type.

In this communication we propose to document more detailed quantitative studies of the general and localized adult differences of the skeleton induced by the dachs gene. Later communications will be devoted to the nature of the microscopic effects and their variations in the course of development.

MATERIALS AND METHODS

The animals described in this study are of New Zealand White origin. All are descendants from one female and her son. Both were heterozygotes *Dada*, and the results of breeding experiments were described earlier. Of the 16 adult skeletons (all at least five and one fourth months of age), seven were homozygous Dachs (*DaDa*), six were heterozygotes (*Dada*), and three normals (*dada*).

Heterozygotes and homozygous "Dachs" were identified at first by breeding test, and later by observation of the abnormal development of the inner margin of the base of the ear (fig. 1). Fifty-three skeletons of other dachs which died or were sacrificed at earlier ages (1–5 months) have also been observed and the developmental information which they supply will be reported later.

The flesh was removed from the skeletons by dermestid beetles.

The skeletons were first examined for the characteristics of chondrodystrophy, and the measurements and illustrations selected are those which seem to critically portray such characteristics as are exhibited by these animals. The measurements are listed

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[2] The authors wish to gratefully acknowledge the technical assistance of DOROTHY KEITH and EUGENE FARRIN.

FIGURE 1.—Shows the characteristic structure of the base of the ear which identifies the homozygous dachs (left), heterozygote (center), and normal (right). The prominent papilla of the homozygote, arising from a broad U-shaped notch at the base of the ear is constant in all individuals, whereas the normal notch is deep and narrow with no indication of a papilla and gives way to the outer margins of the ear at approximately the same level. The notch of the heterozygote is broader and shallower and gives way on one side lower than on the other, with usually but not always a slight prominence reminiscent of the papilla of the homozygote. The variation ranges from one homozygous type to the other with some overlapping of the normal.

and numbered in tables 1, 2 and 3 and portrayed pictorially in figure 3. Terminology is that of BENSLEY (1938).

OBSERVATIONS

The gross external changes, such as enlargement of head, thickening of skin, and prominence of abdomen, so obvious in those chondrodystrophies of other species which are lethal at birth, are not noticeable in the dachs at that age. Except for the ear papilla, previously described (fig. 1), all young from dachs transmitting parents are superficially alike. The *gross external* changes induced by this gene become manifest only after postnatal growth is well established, and as portrayed in the skeleton they fall into two major categories: (a) those features which serve to demonstrate an overall or generalized retardation of body growth and (b) those which by bizarre morphological change, or more subtle quantitative differences, portray the effects of relatively localized differences in growth. Whether these are the results of the simultaneous pleiotropic effects of the gene upon several already differentiating and specifically localized growth processes, or whether in a considerable measure the compensatory or adaptive changes secondary to the initial and primary change or both can be determined only by further study.

Gross influence on body size

As shown in figure 2, the dachs gene does not produce a significant difference in body size during the first 21 days or up to approximately the time of weaning. After that time the difference becomes increasingly greater and based upon analyses of variance is highly significant. After 180 days we do not have sufficient numbers of

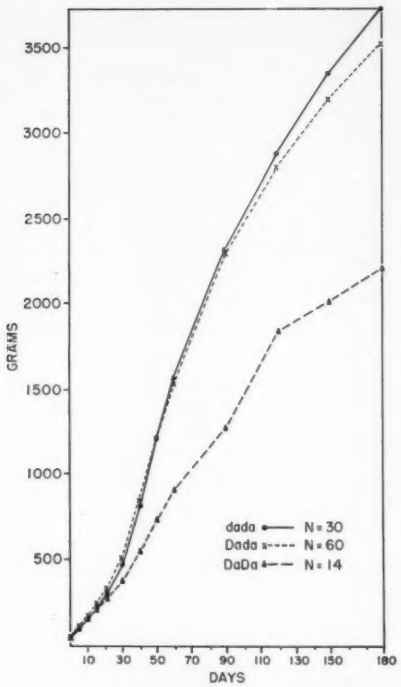


FIGURE 2.—Shows growth curves of body size.

animals for a reliable estimate of the effect of the gene on body weight. Most homozygous individuals become so badly crippled that survival is possible only under the best conditions. Those that do survive lose weight as a result of the handicap. An estimate is provided, however, by the weights of one homozygous dachs female, 1R15, which lived for 21.5 months and produced five litters. They indicate that the effects are not entirely traumatic because although in no case was this mother able to raise her litters, due to her clumsy and micromelic condition, her physical condition remained apparently normal, and several of the litters which also appeared quite normal were fostered successfully. Thus, it seems reasonable to assume that her average weights taken at breeding during the last nine months must be truly representative of the dachs (*DaDa*) constitution. Compared with the average breeding weights of eight heterozygous females similarly bred during that time, she was 850 gms lighter. This limitation in body size by the dachs gene appears greater in males than females, but additional data are needed for reliability.

The Axial Skeleton

SKULL. The typical ***primary*** characteristics of chondrodystrophy, namely the reduction in size and premature fusion of the endochondral bones of the skull, which

are usually manifest in other species, also tend to be present in the dachs rabbit (figs. 6, 7, 8). Their manifestation, however, appears to differ from that usually described. With respect to length, the basioccipital appears to show the least influence of this gene of any bone in the body. Five of the seven dachs skulls have basioccipitals which are equal to or longer than the average normal skull (fig. 6). In the other two (♀ 7G78, fig. 7 and ♀ 5K90) the basioccipital is fused with the basisphenoid, and here the combined length is 1.8 and 1.75 respectively as compared with the average 1.56 for basioccipital and 1.21 for basisphenoid alone. These two animals could not be included in the average figures for the two bones in table 1 (items 4 and 5) because the fusion was complete. Excluding these, the average occipital bone is longer (11.4%) than either the normal or the hybrid, but the difference is not statistically significant.

The presphenoid (item 7, table 1) shows no significant difference between dachs

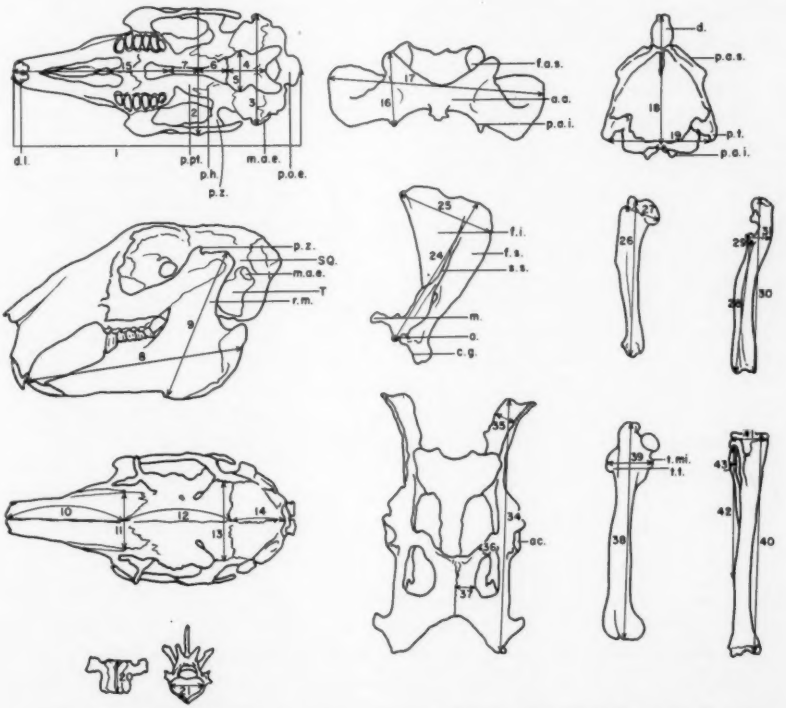


FIGURE 3.—Arrows identify limits of measurements taken. Numbers refer to those in tables 1, 2, and 3. Symbols: a., acromion; a.a., anterior arch of atlas; ac., acetabulum; c.g., glenoid fossa; d., dens; d.i., incisor teeth; f.a.s., superior articular pit; f.i., infraspinous fossa; f.s., supraspinous fossa; m., metacromion; m.a.e., external auditory meatus; p.a.i., inferior articular facet; p.a.s., superior articular facet; p.h., hamular process; p.o.e., external occipital protuberance; p.pt., pterygoid process; p.t., transverse process; p.z., zygomatic process of squamosal; r.m., mandibular ramus; SQ., squamosal; s.s., scapular spine; T., tympanic bulla; t.mi., trochanter minor; t.t., third trochanter.

and normal. This is apparently due to the fact that two sibs, 7G77 and 7G78, have presphenoids of normal length, whereas the other five are significantly shorter (3 mm or 28.5%). The total difference for all is about 18.1%.

The basisphenoid (item 6) is the only of the three which is consistently and significantly shorter (13.6%) than either normal or heterozygote. However, the significance is at the 5% level of probability. Thus, lengthwise the greatest effect on the endochondral bones of the skull is centered in the basisphenoid and tends to affect the presphenoid slightly more than the basioccipital.

When considered transversely, these three bones appear to be consistently reduced as a unit. The presphenoid is so narrow in the rabbit that width is relatively slight, although visibly narrower in the dachs. The basioccipital (item 5, table 1) is significantly narrower in the dachs than in the other two groups, and by inspection at least, the posterior margin of the basisphenoid where it joins the basioccipital is identical with it and therefore may be considered as affected relatively in the same manner.

Thus the Dachs gene appears to exert a relatively greater reduction in width of

TABLE 1

Average measurements, standard deviation, coefficient of variability, and F test of significance between groups

Measurement	*DaDa*			*Dada*			*dada*			Analysis of Variance				
	$\bar{x}$	S.D.	CV	$\bar{x}$	S.D.	CV	$\bar{x}$	S.D.	CV	Percent diff.†	3 groups	*DaDa* vs. *dada*	*DaDa* vs. *Dada*	*Dada* vs. *dada*
1. Skull length	*9.39*	0.38	4.05	*10.33*	0.10	0.97	*9.96*	0.12	1.20	(5.7)	**	*	**	**
2. Skull width at zygomatic arch	*4.33*	0.18	4.16	*4.72*	0.10	2.12	*4.63*	0.46	9.93	(6.5)	**	*	**	—
3. Skull width at auditory meatus	*3.56*	0.16	4.49	*4.0*	0.10	2.50	*3.93*	0.13	3.31	(9.4)	**	**	**	—
4. Basioccipital lgth	*1.56*	0.16	10.26	*1.42*	0.04	2.82	*1.40*	0.05	3.57	(+11.4)	—	—	—	—
5. Basioccipital width	*0.68*	0.04	5.88	*1.02*	0.02	1.96	*1.05*	0.05	4.76	(35.2)	**	**	**	—
6. Basisphenoid lgth	*1.21*	0.11	9.09	*1.37*	0.04	2.92	*1.40*	0.00	0.00	(13.6)	**	*	*	—
7. Presphenoid lgth	*0.63*	0.15	23.81	*0.79*	0.27	34.18	*0.77*	0.12	15.58	(18.1)	—	—	—	—
8. Mandible length	*7.08*	0.28	3.95	*7.97*	0.12	1.50	*7.83*	0.16	2.04	(9.6)	**	**	**	—
9. Mandible depth	*4.78*	0.17	3.56	*5.36*	0.18	3.36	*5.20*	0.17	3.27	(8.1)	*	*	**	—
10. Nasal length	*3.93*	0.18	4.58	*4.25*	0.23	5.41	*3.98*	0.16	4.02	(1.3)	*	—	*	—
11. Nasal width	*1.79*	0.15	8.38	*2.05*	0.10	4.88	*2.05*	0.09	4.39	(12.7)	**	*	**	—
12. Frontal length	*3.58*	0.26	7.26	*3.87*	0.11	2.84	*3.72*	0.20	5.38	(3.7)	—	—	—	—
13. Frontal width	*2.67*	0.16	5.99	*2.66*	0.07	2.63	*2.53*	0.06	2.37	(5.5)	—	—	—	—
14. Parietal length	*1.97*	0.17	8.63	*2.12*	0.13	6.13	*1.98*	0.08	4.04	(0.5)	—	—	—	—
15. Snout length	*4.44*	0.24	5.40	*4.91*	0.11	2.24	*4.58*	0.08	1.75	(3.1)	**	—	**	**
16. Atlas length	*0.91*	0.11	12.09	*1.27*	0.04	3.15	*1.15*	0.08	6.96	(20.9)	**	*	**	*
17. Atlas width	*3.08*	0.11	3.57	*3.59*	0.06	1.67	*3.55*	0.05	1.41	(13.2)	**	**	**	—
18. Axis length	*1.57*	0.12	7.64	*2.18*	0.04	1.83	*2.08*	0.10	4.81	(24.5)	**	**	**	—
19. Axis width	*1.73*	0.11	6.36	*1.84*	0.10	5.43	*1.70*	0.00	0.00	—	—	—	—	—

* = P = <.05
** = P = <.01
† = Change from normal

endochondral skull bones than in length (approximately 35% vs 13–18%) and furthermore in both length and width the influence appears to be considerably localized in its effect.

It now becomes of interest to learn whether these peculiarities are manifest in the rest of the skull, particularly in the larger membrane bones which originate by quite different processes. To obtain this estimate measurements were taken of the overall length of skull and of lengths and widths of certain specific bones as indicated in figure 3 and items 1–3 and 10–15 of table 1. Of these measurements the length and width of the nasal bones are the only ones which are statistically significant. Length of nasal bone is significant only between the dachs and heterozygote at the 5% level of probability. Width of the nasal bone of the dachs is significantly less than in heterozygote or normal. Snout length and overall skull length include the same general area measured by the nasal bones and therefore, as might be expected, are also greater in the heterozygote. This peculiarity in the nasal bones, which is also present in the frontal and parietal bones (although the differences there are not statistically significant), indicates that the effect of the dachs gene upon the membrane bones is not exactly the same as that upon the endochondral bones.

A clue as to its nature is found in study of the lateral aspect of these skulls (figs. 12, 13, 14). As shown in items 8 and 9 of table 1, the dachs mandible manifests the same tendencies to be significantly reduced in both length and depth, although the reduction is a matter of only 8 or 10 percent as compared with 14–18% in the basisphenoid and presphenoid. It is also interesting that they are much larger than the differences found in the other membrane bones which are not significant.

There is one difference, however, which is clearly discernible in the lateral views of figures 6–14. Here it is seen that the entire region at the antero-posterior level of the basisphenoid of the skull of the dachs is very much refined in relation to neighboring parts. This refinement involves the squamosal bone, its zygomatic process, the mandibular ramus and the tympanic bulla, which can be readily seen in figures 13 and 14. It also includes the pterygoid and hamular processes which similarly tend to be reduced. The alteration in size and shape of the bulla seems directly responsible for the marked difference in ear carriage of the living animal (CRARY and SAWIN, 1952), since it is clearly apparent that the bony external acoustic meatus is malformed (laterally compressed).

Associated with these localized changes there are also found changes in the shape of the posterior skull as portrayed in the ventral and lateral views (figs. 6–8, 12–14). There is an obvious lowering of the orbit in its relation to the rest of the skull and a migration backward and downward of the posterior part of the brain case in such a way that the normally posterior margin of the skull containing the occipital complex of bones and the foramen magnum is not vertical as normally but carried at an angle of 30–40°. The foramen magnum thus opens downward rather than posteriorly as in the normal. This is particularly pronounced in those animals such as 7G78 (posterior view fig. 10) in which the basioccipital and basisphenoid are very much reduced and, as previously described, fused. It is less apparent in those such as 3H95 (fig. 11) in which only the basisphenoid is reduced. This also exerts an effect upon the shape of the foramen magnum (figs. 9–11) and other foramena in that region.

Thus, it must have a corresponding effect on the cord and associated nerves and blood vessels. Associated with the more ventral position of the orbit is a depression in the frontal bones which we assume corresponds with that so commonly recognized in other achondroplasias at the base of the nasal bones. These localized skull differences appear to be of special significance in the interpretation of gene influence and will be discussed further.

ATLAS and AXIS. The pattern of changes to be observed in these two vertebrae of the dachs appear to be more closely related with the changes noted in the skull than with those of the remainder of the vertebrae. For that reason they have been included in table 1 (items 16–19) with the skull measurements.

Essentially they are of two kinds, clearly discernible quantitative changes and rather spectacular qualitative changes (figs. 15–23) which obviously modify the methods of achieving the functions which these two vertebrae ordinarily perform.

Quantitatively, length and width of the atlas as they have been measured (items 16 and 17, table 1) are 20.9 and 13.2% less respectively in the dachs than in the normal animals of the race. In the epistropheus (items 18 and 19, table 1) the same differences exist but length is actually more affected (24.5%), whereas width becomes so much less as to be statistically insignificant. Although these width measurements as indicated in figure 3 were taken through the transverse processes whose function is muscle attachment and which therefore could be secondarily influenced by such associated organs, it can be clearly seen that all parts are proportionately nearly the same. Thus, it would seem that the significant primary feature is one of overall size difference in the axis and of length only in the epistropheus.

It is also particularly of interest to note that the heterozygous individuals, as was the case with overall skull length and particularly snout length, are larger in all four measurements than in either homozygous type, although not significantly greater than the normal except in atlas length.

The morphological differences apart from size are not only spectacular but are of special interest as an illustration of a way in which a relatively simple growth deficiency can sometimes disrupt a rather complex normal function and lead to a reestablishment of that function upon a quite different basis. In figures 15–23 are shown ventral and anterior views of the atlas and a ventral view of the axis belonging with the skulls pictured in figures 6–14. They include heterozygote 9N26, dachs 3H95 with long basioccipital to which is attached the dens (fig. 8), and dachs 7G78 with reduced basioccipital fused to basisphenoid and no dens (fig. 7). In the latter case the dens appears as a part of the atlas. The dens in both 3H95 and 7G78 being absent from the axis as is the case in all dachs (interpretation based upon presently described material and 53 additional homozygous dachs over one month of age), the superior articular facets approximate and join both anteriorly and ventrally with each other for a more or less continuous articulating surface. This apparently permits the side to side movement of the head, normally possible by rotation of the atlas with the skull. In 7G78 the superior articular pit is extremely reduced and almost nil, the articulation appearing very nearly to approach a fusion. Such movement as is normally possible by this joint appears to have been transferred more effectively to the joint formed by the inferior articular facet of the atlas and the superior articular facet of the axis.

Not only is the position and attachment of the dens altered from the normal but there are further changes in the ventral portion of the atlas. In both 7G78 and 3H95, and in the homozygous dachs generally, the ventral arch of the atlas is not completely fused with the transverse processes so that the intervening sutures can be clearly seen (figs. 16, 17, 19, 20). Within the ventral arch in most cases there are found a number of small bony elements of varying sizes, shapes and degree of fusion. In some cases the ventral arch consists of two major parts which suggest the bipartite origin of the centrum observed by BEADLE 1931, NOBACK 1944, and SAWIN and CRARY 1956. These observations together with the significant reduction in size are indicative of the location and nature of the retardation in growth which has taken place and also perhaps of the time at which it has occurred.

Further it is of interest to note the effect which these primary changes in growth and development have had upon the flexion-extension and rotation movements of the skull. The former movement, which normally is a function of the occipital condyles of the skull and superior articular pits of the atlas, in most cases becomes partially transferred to the joint of atlas and epistropheus.

The latter bone, unhampered by the dens, thus has a greater freedom of relation with the atlas, and the joint between them, as indicated by the articular surfaces previously mentioned, assumes the function normally performed more anteriorly.

VERTEBRAE (3–31). The measurements for these vertebrae are shown graphically

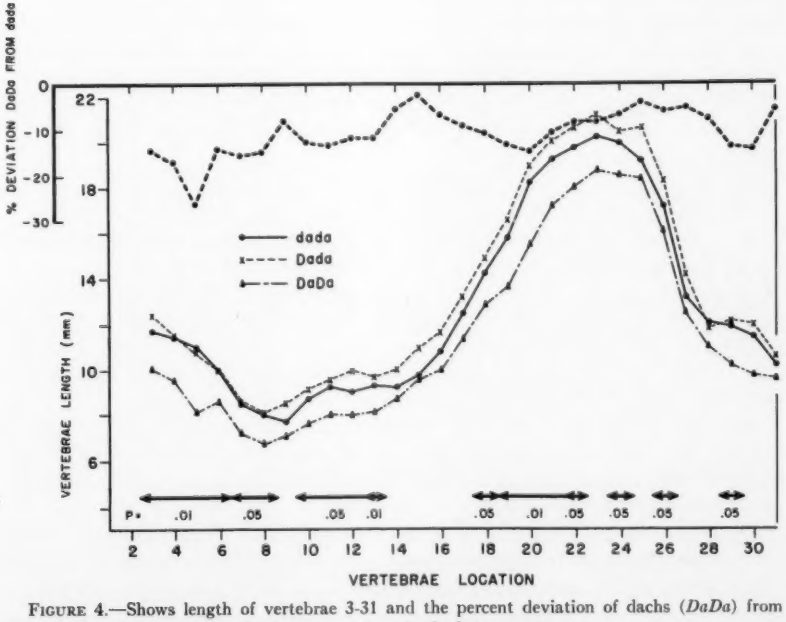


FIGURE 4.—Shows length of vertebrae 3-31 and the percent deviation of dachs (*DaDa*) from normal. Note the greater length of most vertebrae in the heterozygote.

in figures 4 and 5 (see fig. 3 for position of measurements). Two characteristics appear to be significant. First, gross inspection of all the vertebrae as a group gives the impression of a generalized refinement of each vertebra throughout the column of the dachs. By actual measurement lengths of the vertebrae (fig. 4) are significantly less than in the normal for all vertebrae except 9, 14–17, 23, 25, 27, 28, 30 and 31. Of these, vertebrae 27 and 28 are transitional vertebrae between lumbar and sacral regions and 30 and 31 between the sacrum and the tail in which there is normally a considerable variation in size depending upon whether the coxa is attached to the 27th or 28th vertebra or in some measure upon both. In this particular population it so happens that there are seven animals with 26 presacral vertebrae, seven with 27 and two in which the coxa is attached assymetrically to both the 26th and the 27th. Thus, these exceptions are due to an entirely different and recognizable cause. Explanations for the lack of significant reduction in length of the other seven vertebrae above becomes apparent in discussion of the localized differences below.

Since all other cases are significantly less at least at the 5% level, and most cases at the 1%, the dachs gene when homozygous appears to have induced a generalized reduction in the length of most vertebrae, as well as in the length of the skull, but with some tendency to localize anteriorly.

Second, in the heterozygous condition there is a generalized increase in length of all vertebrae over that of the normal which corresponds to increases noted in the length of the skull and snout. *They are significantly greater than the normal*, however, in only two cases, namely, vertebrae 15 and 25, and then only at the 5% level. In view of the small numbers of normal rabbits on which these figures are based, no great importance can be attached to them at this time except as they seem to portray a general size difference. A repeated and significant difference of this kind induced by one pair of genes would be of considerable interest genetically in connection with the phenomenon of overdominance, and this is being studied at present in material obtained at younger ages.

Differences in width of the vertebral bodies (fig. 5) between dachs and normals are relatively much less than those in length and are only significant in the lumbar area, vertebrae 22–26, and are reminiscent of the more localized width differences of the skull, atlas, and axis. There is a slight tendency for some of the vertebrae in the dachs to be wider than those in the normal, and vertebrae 16, 29 and 31 are significantly so, at least at the 5% level. Of these vertebrae, 16 and 31 are of the group showing no significant reduction in length. Thus, the overall size of these two vertebrae is relatively increased. In only one place, at the 31st vertebra, is the width of the vertebrae of heterozygotes significantly greater than the normal.

It is also interesting to note the presence of small, independent or partially fused traction epiphyses at the tips of many of the vertebral processes (spinous, mamillary, and particularly transverse) of the dachs. The ventral spinous processes do not show these, but appear to be longer than in the normal.

Paralleling the generalized refinement of the vertebrae is a general tendency in the lumbar area for the transverse processes to be restricted in their spread and angle at which they project laterally and ventrally, as indicated by both general appearance and actual measured distance from tip to tip, and there is greater variability than in

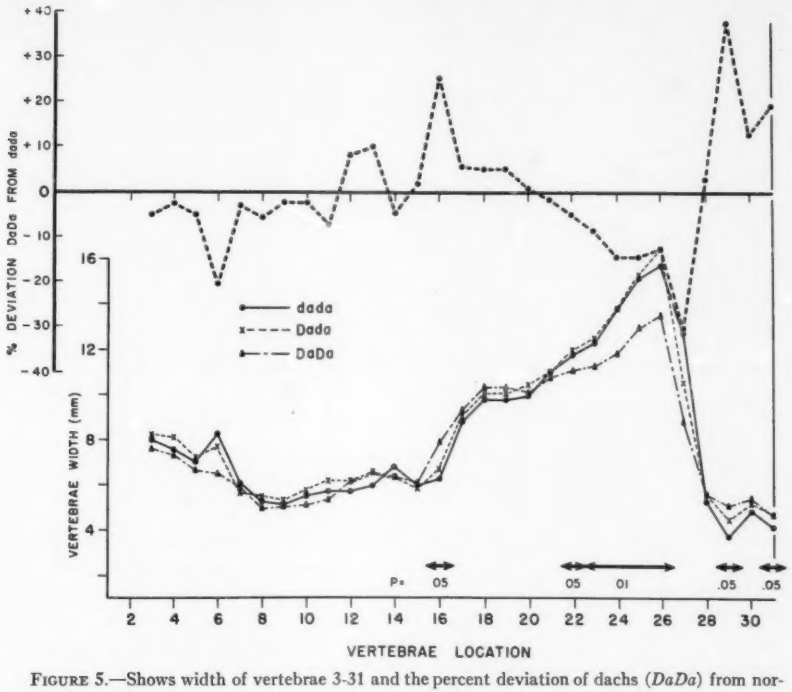


FIGURE 5.—Shows width of vertebrae 3-31 and the percent deviation of dachs (*DaDa*) from normal. Note positive deviations of the dachs whereas lengths (fig. 4) were all negative.

width of centra. For instance, in the cervical and transitional thoraco-lumbar vertebrae there is a noticeable tendency for the transverse processes to be shorter. The difference is highly significant particularly on vertebrae 10, 20 and 23–27.

In heterozygotes the distance between distal ends of the transverse processes are significantly greater than in either homozygote from vertebrae 6–15, but in the lumbar region the heterozygotes are intermediate between the homozygous groups. They are significantly smaller than the normal on vertebra 20, but significantly larger than the dachs on vertebrae 23–27.

Thus, although there is a general tendency to refinement in the dachs, it is apparent that the pattern of development transversely differs from that longitudinally, and it also differs in the transverse processes as compared with the vertebral bodies. There seems to be no evidence of any competitive relationship between length and width.

Appendicular skeleton

The bones of the appendicular skeleton all show considerable reduction in the dachs, varying from 15.4% to as much as 45% as compared with the skull and vertebrae in which the differences nowhere were greater than 35% and most of which were

TABLE 2

Average measurements of long bones of fore limb

Measurement		*DaDa*			*Dada*			*dada*			Analysis of Variance			
		$\bar{x}$	S.D.	CV	$\bar{x}$	S.D.	CV	$\bar{x}$	S.D.	CV	3 groups	*DaDa* vs. *dada*	*DaDa* vs. *Dada*	*Dada* vs. *dada*
Clavicle														
22	length	*2.26*	0.24	10.91	*2.64*	0.15	5.68	*2.60*	0.13	5.00	*	*	*	—
23	width	*0.29*	0.00	0.00	*0.20*	0.00	0.00	*0.20*	0.00	0.00	—	—	—	—
Scapula														
24	length	*4.93*	0.37	7.50	*7.24*	0.22	3.04	*7.50*	0.25	3.33	**	**	**	*
25	width	*2.43*	0.45	18.52	*4.15*	0.23	5.54	*4.42*	0.15	3.39	**	**	**	—
Humerus														
26	length	*6.37*	0.30	4.71	*7.82*	0.29	3.71	*8.20*	0.29	3.54	**	**	**	—
27	width	*1.63*	0.09	5.52	*1.73*	0.06	3.47	*1.67*	0.07	4.19	—	—	—	—
Radius														
28	length	*5.61*	0.15	2.67	*7.06*	0.22	3.12	*7.70*	0.08	1.04	**	**	**	**
29	width	*0.86*	0.04	4.65	*0.90*	0.06	6.67	*0.83*	0.03	3.61	—	—	—	—
Ulna														
30	length	*6.70*	0.18	2.69	*8.45*	0.26	3.08	*9.06*	0.15	1.65	**	**	**	**
31	width	*1.09*	0.05	4.59	*1.18*	0.08	6.78	*1.05*	0.05	4.76	*	—	*	*
3rd metacarpal														
32	length	*1.64*	0.08	4.88	*2.21*	0.13	5.88	*2.52*	0.08	3.17	**	**	**	**
33	width	*0.30*	0.00	0.00	*0.30*	0.00	0.00	*0.30*	0.00	0.00	—	—	—	—

less than 25% as compared with the normal. Furthermore, they show a considerable amount of rotation, bowing and frequently contortion, and the cavities for articulation, especially the glenoid fossa and acetabulum, are malformed and surrounded frequently by several small exostoses.

FORE LIMB. The measurements of lengths and widths of the fore limb bones are shown in table 2, and photographs of typical examples are shown in figures 24–28. The clavicle (items 22 and 23, table 2) shows a reduction of 15.4% in length for the dachs over that of the normal, but differences in width are negligible. Of all the bones of the body the scapula shows the greatest differences and greatest variability in the measurements taken (figs. 24–26). In the dachs it is small, twisted and contorted. The glenoid fossa is broad and shallow and the spine thickened and curved. The supra- and infraspinous fossae are also narrowed and shallow. The acromion and metacromion in some cases appear relatively normal and in others much reduced. The entire bone shows evidence of twisting and contortion.

By measurement the scapula is both shorter (34.3%) and particularly narrower (45.0%), and even in the hybrid length is significantly less than in the normal. It is the only bone of the fore limb to show a significant reduction in width.

In the humerus, radius, and ulna the lengths are significantly reduced in the dachs, and, as shown in figures 27 and 28, there are obvious evidences of rotation bending and contortion. Although the proximal humerus of the dachs rabbit may appear relatively enlarged as is considered typical of chondrodystrophies, in this case it appears to be an illusion resulting from the relative shortening. In one or two cases thickening of the ulna is apparent, as in 3H95, but this we believe is a secondary result associated with the bowing of the limb, and will be discussed later.

The metacarpals (items 32 and 33, table 2) likewise show the same significant differences in length but not in width.

TABLE 3

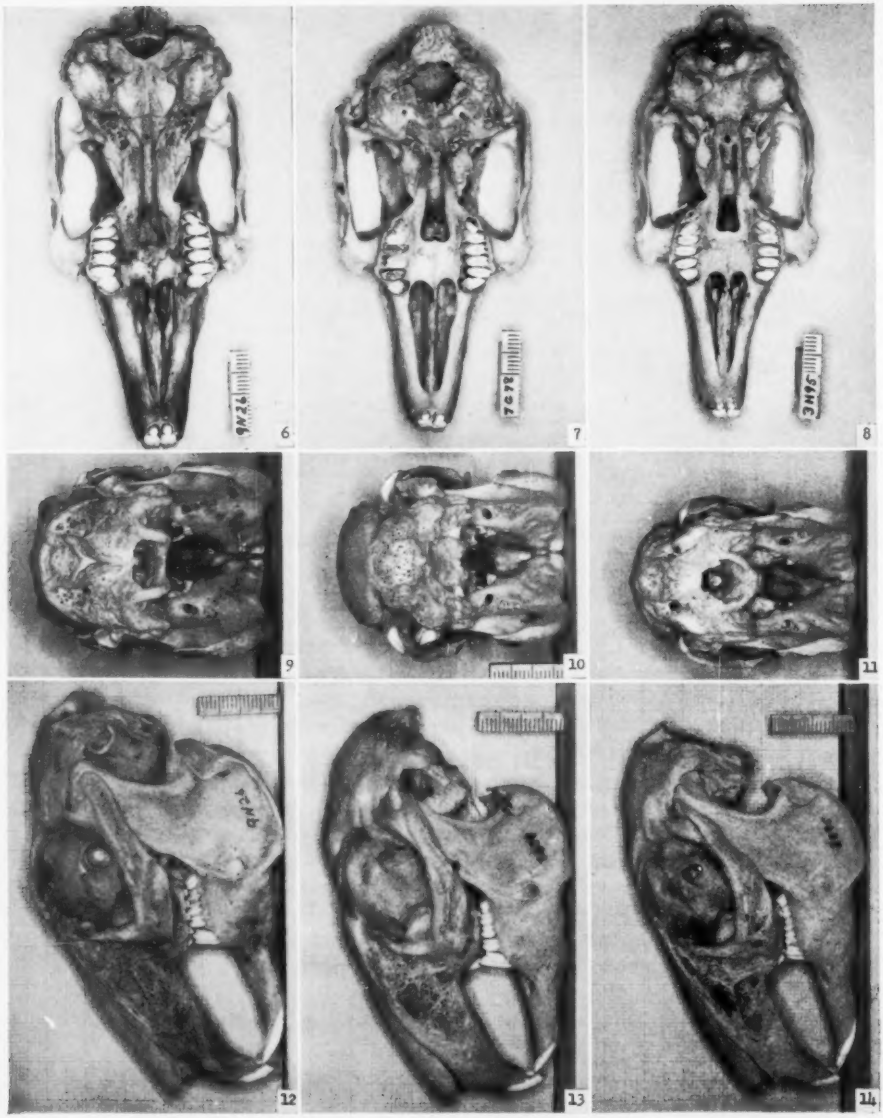
Average measurements of long bones of hind limb

Measurement		*DaDa*			*Dada*			*dada*			Analysis of Variance			
		$\bar{x}$	S.D.	CV	$\bar{x}$	S.D.	CV	$\bar{x}$	S.D.	CV	3 groups	*DaDa* vs. *dada*	*DaDa* vs. *Dada*	*Dada* vs. *dada*
Iliac wing														
34	length	*8.62*	0.61	7.08	*10.15*	0.23	2.27	*10.15*	0.35	3.45	**	**	**	—
35	width	*1.76*	0.22	12.50	*2.24*	0.15	6.70	*2.30*	0.18	7.83	**	**	**	—
Os coxa														
36	width body of pubis	*0.54*	0.05	9.26	*0.63*	0.08	12.70	*0.67*	0.10	14.92	*	*	*	—
37	width infra ramus	*0.44*	0.07	15.91	*0.80*	0.20	25.0	*0.57*	0.18	31.58	**	—	**	—
Femur														
38	length	*8.55*	0.35	4.09	*10.37*	0.29	2.80	*10.85*	0.35	3.22	**	**	**	—
39	width	*1.79*	0.11	6.14	*2.22*	0.08	3.60	*2.15*	0.42	19.53	**	**	**	—
Tibia														
40	length	*9.14*	0.26	2.84	*10.90*	0.30	2.75	*11.63*	0.20	1.72	**	**	**	**
41	prox. width	*1.93*	0.11	5.70	*1.94*	0.08	4.12	*1.87*	0.03	1.60	—	—	—	—
Fibula														
42	length	*3.36*	0.24	7.14	*4.26*	0.31	7.28	*4.98*	0.19	3.81	**	**	**	**
43	width	*0.53*	0.09	16.98	*0.61*	0.04	6.56	*0.55*	0.05	9.09	—	—	—	—
3rd metatarsal														
44	length	*3.28*	0.14	4.27	*4.05*	0.13	3.21	*4.43*	0.11	2.48	**	**	**	**
45	width	*0.41*	0.02	4.88	*0.41*	0.02	4.88	*0.43*	0.03	6.95	—	—	—	—

HIND LIMB. In the coxa (see items 34–37, table 3) the differences in both length and width in the dachs are closely similar to those in the scapula. Actual distortion of the pelvis, except for the acetabular area is less obvious than in the scapula (figs. 29–31). Both length and width are significantly less in the dachs than in the normal, which may well be a manifestation either of the general reduction in size of the entire bone complex (as for example in 3H95, fig. 31), or it could be a result of deficient muscular development secondarily arising from the abnormal acetabulum and resulting in crippling and lack of use of the limb. These possibilities can be determined only by studying the development of young.

As in the fore limb, the long bones all show significant reduction in length (items 38, 40, 42 and 44, table 3). Width of femur as taken from the minor and third trochanters is significantly less. Widths of the other long bones are not significantly different. This results from the change in shape of the proximal femur associated with the above mentioned dislocation (3H95, fig. 32). The distal articular area of the femur and proximal tibia also appear enlarged as portrayed in figures 32 and 33. Although this again may be the same illusion referred to in the fore limb, close examination of the condyles and fossae in some cases reveals obvious signs of trauma and thus it seems altogether likely that in such cases at least there is a real but slight enlargement. This might be explained as a secondary result of the abnormal pressures occasioned by the earlier dislocation of the femur. However, close scrutiny also reveals the same tendencies to rotation, bending and contortion observed in the fore limb.

The fact that the hyperplasia in the region of the metaphyses which results from disproportionate growth, usual in other species, is not generally indicated here in any of the long bones may be a result of the advanced age of these skeletons (WARKANY, 1954), or it may be due to our method of skeleton preparation by dermestid

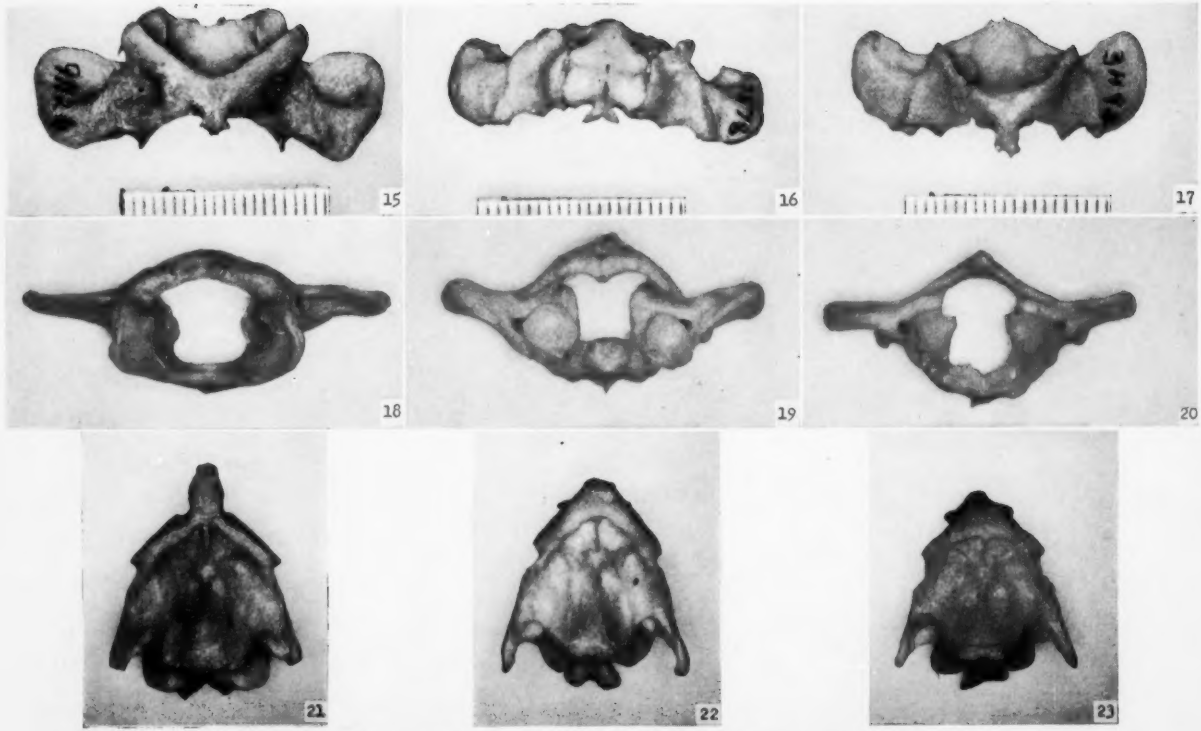


FIGURES 6–14—Lateral, posterior, and ventral views of the skull.

FIGURES 6, 9, 12. Heterozygous.

FIGURES 7, 10, 13. Homozygous dachs with fused basioccipital and basisphenoid.

FIGURES 8, 11, 14. Homozygous dachs with normal basioccipital and attached dens.

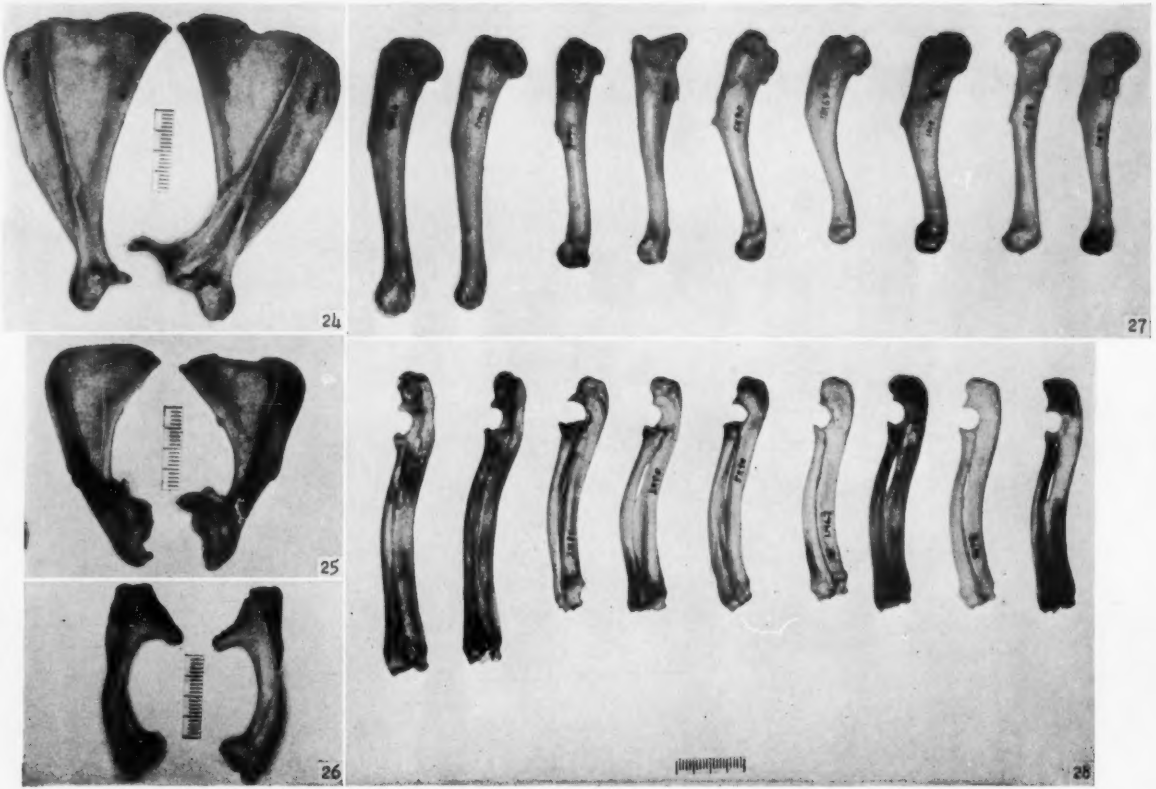


FIGURES 15–23—Views of atlas and axis (for symbols see fig. 3).

FIGURES 15, 16, 17. Ventral views of atlas of same animals as in figures 6–14, f.a.s. at the top. Note the attached dens on figure 16 and failure of fusion of anterior arch (a.a.) in both figures 16 and 17.

FIGURES 18, 19, 20. Anterior aspect of atlas of the same animals, showing normal superior articular pits, and as modified in the same dachs animals with dens present (fig. 19) and absent (on the basioccipital) (fig. 20).

FIGURES 21, 22, 23. Ventral view of the epistropheus of the same animals, showing absence of dens in dachs animals (figs. 22 and 23).

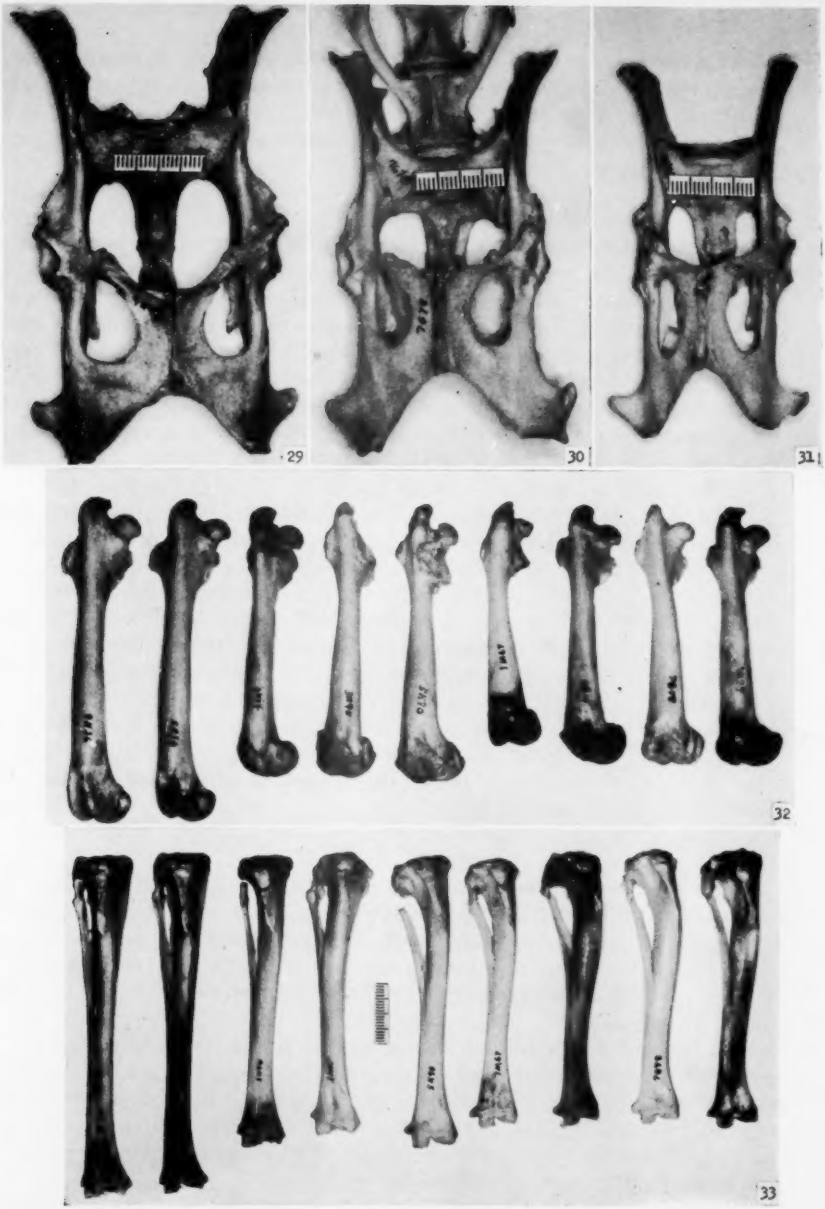


FIGURES 24–28—Views of bones of the fore limb.

FIGURES 24, 25, 26. The scapulae of the same three animals of figures 6–14 and 15–23.

FIGURE 27. The humeri of two heterozygotes and seven dachs showing relative size, rotation and distortion.

FIGURE 28. Same for the radii and ulnae.



FIGURES 29–33—Views of bones of the hind limb.

FIGURES 29, 30, 31. The pelves of the same three animals of figures 6–14 and 15–23.

FIGURE 32. Femurs of the same group of animals as in figures 24–28 in same order.

FIGURE 33. Tibiae and fibulae of the same group of animals as in figures 24–28 in same order. Again note relative size, rotation and distortion of the long bones and also the defective acetabulae and heads of the femurs (see text).

beetles in which there is opportunity for loss of persistent cartilage. It is being investigated further in histological and developmental studies now in progress. On the other hand, other investigators have compared length and width in relation to each other and by this means our material also exhibits a greater width. Hence hyperplasia would seem to be more apparent than real.

DISCUSSION

The homozygous dachs manifests certain of the typical characteristics of chondrodystrophy but there are certain unique differences. Diminished size and shortening of the limbs appear to be consistent but variable in their expression. Hyperplasia of cartilage in the region of the metaphysis and any profound enlargement of the head seem to be absent except as they may be secondary manifestations or are mildly expressed by individual bone measurements. The depression at the nasion appears to have migrated posteriorly and is accompanied by depression and shift of the orbit and optic foramen. Associated with these are highly localized disproportionate differences which are unlike those noted in any other inherited chondrodystrophy thus far described.

Chondrodystrophies described in the literature s ⊥ to fall into two categories: those which are lethal when homozygous (whether dominant or recessive) and those which are not. In those most thoroughly studied, the effects of the gene upon the morphological development of both limb and skull are extreme, and death occurs prepartum or very early postpartum. In the second group the effects are less severe, and apparently become grossly manifest much later, even considerably after birth, and there is a greater variety of expression. Some of them are sufficiently compatible with normal life that they have by selective breeding become characteristics of recognized and established breeds of animals. In the basset hound and dachshund the legs appear to be the only parts affected. In the bulldog the legs, although short, are relatively unaffected, but the base of the skull and tail show a marked degree of chondrodystrophy (STOCKARD 1941). In the Ancon sheep the skull is only mildly affected and the limbs are not only short but the effect is localized distally (CHANG 1949).

With respect to these the dachs rabbit appears to be intermediate between the Ancon sheep and bulldog insofar as localization of effects is concerned. Its major and most obvious effect and its generalized reduction in size is accentuated in the limbs, particularly proximally in the girdles and distally in the feet. The major effects upon the axial skeleton anteriorly not only include the skull but also the first two vertebrae.

Since the usual hyperplasia in the regions of the metaphyses tends to be absent, width does not appear to be secondary to defective development of columnar cartilage as interpreted by PEARCE and BROWN (1945a) and WARKANY (1954) for other chondrodystrophies. In fact, the homozygous dachs (*DaDa*) is characterized by notable differences in both lengths and widths which tend to be strongly localized and relatively independent of each other.

For example, the relatively unaffected length of the basioccipital is accompanied by a reduction in width, a characteristic which is rarely mentioned in other species.

CHANG (1949) noted that the occipital condyles of the Ancon sheep tended to become approximated, but in the dachs the width of the entire bone is reduced, and associated with it is a corresponding change in width of the atlas but not in the axis.

These are followed by significant reductions in size of the succeeding vertebrae, in which the lengths of vertebrae tend to be significantly affected anteriorly (cervical and thoracic) and the widths posteriorly (in the lumbar region). The most significant differences in both length and width of skeletal units are localized in the limbs, and it is of interest to note that by comparison with the vertebrae the long bones are all significantly reduced in length. With respect to width, however, the significant effects occur only proximally. Thus not only are the patterns for length and width in general dissimilar but likewise the pattern of the axial skeleton from anterior to posterior is not comparable regionally with that of the limb proximo-distally for either length or width of skeletal unit.

However, when widths of axial units are compared with lengths of appendicular units there is a striking similarity of pattern in that both manifest two regions of significant reduction of growth (widths of basioccipital and vertebrae 1 and 2 and the lumbar vertebrae *vs.* lengths of girdles and metacarpals) separated by regions manifesting relatively lesser differences (width of cervical and thoracic vertebrae and length of the long bones of the limbs). Thus the pattern of longitudinal growth in the limb (proximo-distally) appears to reflect the transverse growth (anteriorly to posteriorly) in the axial skeleton.

Although the patterns are different, the same sort of relationship is also manifest in the heterozygotes (*Dada*). Here it becomes apparent that the length of parts of heterozygotes is increased locally above that of either homozygote in those regions in which the differences between homozygotes are relatively small, as is the case of length of vertebrae; whereas when differences are relatively larger, as in width of vertebrae, the heterozygotes tend to be intermediate. In the limbs where differences in widths of homozygotes are small, the measurements of heterozygotes tend to be greater, although not significantly so, but lengths are intermediate and significantly different. Thus, although the pattern of regional effect of the dachs gene upon either axial or appendicular skeleton is not exactly the same as in other species, the similarity of pattern in both directions implies a generalized effect which becomes accentuated either at a critical time, as suggested by STOCKARD (1921, 1941), or perhaps more precisely in those units which normally grow the most rapidly (LANDAUER 1931), possibly in both. Since in one region of the dachs at least, in the atlas, axis and occiput, we have evidence of both very early (migratory tendencies of the dens) and very late (failure of secondary centers of ossification to unite with the arch even in adult) retardation, it would seem that the effects of the dachs gene are more nearly in harmony with LANDAUER's interpretation. It would seem difficult to explain them solely on the basis of a single tissue difference, either a defect in columnar cartilage (PEARCE and BROWN 1945a; WARKANY 1954) or endochondral *vs.* periosteal ossification (COWDRY 1946; CREW 1923; KNÖTZKE 1929).

At the same time with respect to the depression in the frontal bones (instead of the nasion) the changes in position and shape of the orbit, optic foramen and external auditory meatus and carriage of the ears, these all appear comparable to changes

reported in other chondrodystrophies. The differences in their manifestation can be explained by the secondary response of the skull units involved to the change in locale at which the retardation is focused, i.e. in the sphenoid rather than the entire endochondral complex. Here again in this region the breadth of the mandibular ramus, zygomatic process, and meatus of the heterozygote visibly matches the overgrowth in other regions (length of vertebrae and skull and snout length, table 1) noted above. These hybrid differences when larger than either parent are referred to by geneticists as manifestations of overdominance (CROW, *et al.* 1952). However, variation in its expression from one region to another, insofar as we know, is unique in this case and merits further study.

Whether the regional differences here observed are an effect of the dachs gene upon the areas growing most rapidly at one critical time which persists, or whether they are due to the interaction of this gene with regional factors inherent within the race needs further investigation. Such regional differences are known to occur in the rabbit (CRARY and SAWIN 1955) independently of both the *Da* and the *ac* (lethal achondroplasia, BROWN and PEARCE 1945; PEARCE and BROWN 1945a, 1945b) genes.

Evidence of regional action of other chrondrodystrophies is apparent in the Creeper fowl as observed by LANDAUER in which the hind limb is influenced the most and in man where KNÖTZKE found the vertebrae of the lumbar region of chondrodystrophies broader than normal. In the dachs it is significant that the greater influences are in the fore limb and, with regard to width of vertebrae, in the posterior thoracic region. Thus it appears that in the rabbit we have regional growth differences which in one case are racial and in the other are due to these specific genes for chondrodystrophy. The use of both of these in hybridization studies would appear to offer valuable tools for study of normal and abnormal growth processes, both for the specific gene activity in the etiology of chondrodystrophy and for various types of growth pattern induced by modifying factors, and for the phenomenon of overdominance.

SUMMARY AND CONCLUSIONS

Evidence obtained from mean body weights from birth to 180 days and quantitative study of 16 adult skeletons of homozygous dachs, heterozygotes and normals within the parental New Zealand White race shows that the dachs gene (*Da*) is responsible (1) for a significant generalized reduction in the size of the body in the homozygote and to a lesser degree in the heterozygote, and (2) for significant localized effects upon the endochrondral bones of the skull, the limb girdles, the atlas and axis, and to a lesser extent in the other vertebrae and long bones. Accompanying them are obvious quantitative reductions in the posterior skull and qualitative changes in the position of the dens, and in shape and rotation of the long bones and their articulations which are considered to be secondary and adaptive. Depression of the nasion, contour of the skull, and relative increase in width of the long bones, particularly that associated with hyperplasia in the region of the metaphysis (characteristics of typical chondrodystrophies of other species) are either absent or shifted in their relative position. The significant differences in the patterns of longitudinal and transverse growth of the various skeletal units, as compared between dachs and normal and particularly between homozygote and heterozygote (including overdominance) con-

stitute irregularities of considerable interest in the final interpretation of the basic gene influence. It would seem difficult to explain them solely on the basis of a defect in columnar cartilage or endochondral *vs.* periosteal ossification, interpretations offered for other chondrodystrophies, and an alternative hypothesis in terms of regional growth is suggested. As indicated by the shift in position of the dens, which must be determined relatively early embryologically, and the failure of fusion of accessory centers of ossification of the atlas in adults, an extended or persistent duration of effect of this gene is implied.

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